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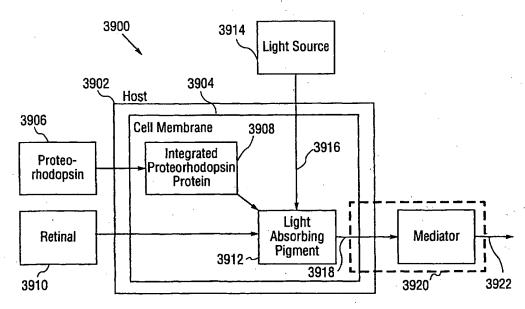
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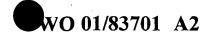
(54) Title: LIGHT-DRIVEN ENERGY GENERATION USING PROTEORHODOPSIN



(57) Abstract: A light-driven energy generation system using proteorhodopsin is provided. Proteorhodopsin sequences were retrieved and amplified from naturally occurring members of the domain Bacteria using proteorhodopsin-specific polymerase chain reaction primers. Proteorhodopsin sequences were placed in expression vectors for production of proteorhodopsin proteins in a host, for instance, *E. coli* and other bacteria. The system also includes a light source and a source of retinal, that allows the system to convert light into biochemical energy. The generated biochemical energy could be mediated into electrical energy by a mediator.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

#### PATENT APPLICATION

# LIGHT-DRIVEN ENERGY GENERATION USING PROTEORHODOPSIN

#### **INVENTORS**

### Edward F. DeLong and Oded Beja

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is cross-referenced to and claims priority from U.S Provisional application 60/201,602 filed 05/03/2000, which is hereby incorporated by reference.

### STATEMENT REGARDING FEDERALLY SPONDORED RESEARCH OR DEVELOPMENT

This invention was supported in part by grant number OCE 0001619 from the National Science Foundation (NSF). The U.S. government has certain rights in the invention.

# STATEMENT TO COMPUTER DISK AND SEQUENCE LISTING

This application includes a sequence listing of 65 sequences and a computer disk labeled "Sequence Listing for application entitled "Light-driven energy generation using proteorhodopsin" by Edward F. DeLong and Oded Beja" containing files "MBA101-SEQLIST.prj", dated "04/23/01" with 174,089 bytes, which is the PatentIn

project file generated using PatentIn Version 3.0 software provided by the USPTO, and "MBA101-SEQLIST.txt", dated "04/23/01" with 323,739 bytes, which is the generated sequence listing from the PatentIn project file MBA101-SEQLIST.prj using PatentIn Version 3.0 software, all which are herein incorporated. The information recorded in computer readable format on the incorporated computer disk labeled "Sequence Listing" containing files "MBA101-SEQLIST.prj" and "MBA101-SEQLIST.txt" are identical to the incorporated written sequence listing.

#### FIELD OF THE INVENTION

The present invention relates generally to gene expression of functional recombinant proteins in bacteria. More particularly, the present invention relates to proteorhodopsin genes and proteins that function as a light-driven energy generator in *Escherichia coli* (E. coli) and other bacteria.

#### **BACKGROUND ART**

Retinal (vitamin A aldehyde) is a chromophore that binds integral membrane proteins (opsins) to form light-absorbing pigments called rhodopsins. Rhodopsins are currently known to belong to two distinct protein families. The visual rhodopsins, found in the eye throughout the animal kingdom, are photosensory pigments. Archeal rhodopsins, found in extreme halophilic environments, function as light-driven protons pumps (bacteriorhodopsins), chloride ion pumps (halorhodopsins), or photosensory receptors (sensory rhodopsins). The two protein families show no significant sequence similarity and may have different origins. They do, however, share identical topologies characterized by seven transmembrane  $\alpha$ -helices that form a pocket in which retinal is covalently linked, as a pronated Schiff base (helix G).

The archaeal rhodopsins are able to generate a photocycle which produces a chemiosmotic membrane potential in response to light, as such light energy is converted into biochemical energy. Recently, a protein with high sequence similarity to the archaeal rhodopsins has also been retrieved in the eukaryote Neurospora crassa (J.A. Bieszke et al., Proceedings of National Academy of Sciences USA 96:8034, 1999). The eucaryal rhodopsin formed a photochemically reactive pigment when bound to all-trans retinal and exhibited photocycle kinetics similar to those of archaeal sensory rhodopsins (J.A. Bieszke et al., Biochemistry 38:14138, 1999). To date, however, no rhodopsin-like sequences have been reported in members of the domain Bacteria, and no light-driven proton pumps based on rhodopsin have ever before been functionally expressed in E coli.

The phototropic conversion of light energy into biochemical energy using bacteriorhodopsin can be harnessed for a variety of processes and applications, such as bio-electronic applications and bio-materials, as has been reported in US Patent No. 5,757,525 for optical devices, US Patent No. 5,854,710 for optical Fourier processing, and US Patent No. 5,470,690 for optical information storage. Bacteriorhodopsin in bio-electronic applications is aimed to interface, integrate, or substitute the silicon based microelectronics systems as well as molecular devices. Bacteriorhodopsin as a bio-material is integrated, for instance, in optical films for light mediated computer memory applications and pattern recognition.

Prevsiously, archaeal rhodopsins capable of generating a chemiosmotic membrane potential in response to light had only been found in halophilic archaea. Therefore, rhodopsins that originate from archaea adapted to highly saline environments cannot be functionally expressed in *E. coli*. Finally, the isolation and cultivation of

halorhodopsins is an elaborate process. At present one does not foresee an economic utilization possible for this process (e.g. US Patent 5,290,699).

Accordingly, as one skilled in the art might readily acknowledge, there is a strong need to retrieve and provide rhodopsin-like sequences from naturally occurring members of the domain Bacteria.

#### **OBJECTS AND ADVANTAGES**

In light of the above, it is the primary objective of the present invention to provide rhodopsin-like sequences from naturally occurring members of the domain Bacteria. More specifically, it is the objective of the present invention to provide a method to retrieve proteorhodopsin genes from DNA of naturally occurring bacteria that encodes DNA sequence for proteorhodopsin proteins.

It is another objective of the present invention to provide proteorhodopsin-specific polymerase chain reaction primers that amplify the proteorhodopsin-containing gene from a DNA sample of naturally occurring bacteria.

It is yet another objective of the present invention to produce variants of a proteorhodopsin gene using the same proteorhodopsin-specific polymerase chain reaction primers by amplifying a proteorhodopsin-containing gene from of a mixed sample of naturally occurring bacteria.

It is still another objective of the present invention to provide an expression vector that produces a proteorhodopsin protein in *E. coli* and other bacteria.

It is another objective of the present invention to provide a light-driven energy generator in which the functional properties of proteorhodopsin are utilized. These properties include the ability to integrate within a host, for instance a cell membrane of *E. coli*, making an integrated proteorhodopsin protein, and the ability to bind retinal, making a light absorbing pigment.

It is another objective of the present invention to provide a light source and illuminate the light absorbing pigment to convert light energy into biochemical energy.

It is another objective of the present invention to provide a mediator and mediate the biochemical energy into electrical energy.

It is another objective of the present invention to provide methods to manipulate the kinetics of the light-driven energy generator.

The advantage of the present invention over the prior art is that it is not restricted to operate in halophilic archaea and could therefore be functionally expressed in *E. coli* and other bacteria. Accordingly, another advantage of the present invention is that it provides for a fast and cheap production method that allows for mass production of functionally active proteorhodopsin.

#### SUMMARY

The present invention provides proteorhodopsin gene and protein sequences retrieved from samples of naturally occurring members of the domain Bacteria. More specifically, the present invention provides a method for the retrieval and amplification of proteorhodopsin genes from DNA samples of naturally occurring marine bacteria. In accordance with several exemplary embodiments of the present invention, DNA samples were obtained from naturally occurring bacteria such as, for instance, marine proteobacteria, SAR86 bacteria, or recombinant DNA libraries containing naturally occurring bacteria. The present invention provides proteorhodopsin-specific polymerase chain reaction (PCR) primers to amplify a proteorhodopsin gene from DNA samples of these marine bacteria. The present invention also provides a device and method for the placement of proteorhodopsin genes in an expression vector to produce functional proteorhodopsin proteins in *E. coli* and other bacteria.

Accordingly, the present invention provides a method to produce and obtain variants of proteorhodopsin genes and proteins. The same proteorhodopsin-specific polymerase chain reaction primers amplify different variants of proteorhodopsin-containing genes from a mixed sample of naturally occurring bacteria. As one skilled in the art might readily acknowledge, these variants of a proteorhodopsin gene produce functional variations in the photocycle kinetics of the proteorhodopsin protein.

Furthermore, the present invention provides a light-driven energy generator that utilizes proteorhodopsin to convert light-energy into biochemical energy. This light-driven energy generator takes advantage of the functional properties of the proteorhodopsin protein once expressed in, for example, *E. coli* or other bacteria as is

described in exemplary embodiments. These properties include the ability to integrate within a host such as, for instance, a cell membrane of *E. coli* or other Bacteria, and thereby making an integrated proteorhodopsin protein or integrated cell membrane protein. These properties also include the ability to bind retinal and thereby making a light absorbing pigment. Illuminating the light absorbing pigment with a light source converts light energy into biochemical energy. Finally, the biochemical energy can be mediated into electrical energy by a mediator.

In accordance with exemplary embodiments, the present invention enables one skilled in the art to manipulate the kinetics of the proteorhodopsin protein photocycle once it is operational in the light-driven energy generator. In particular, the present invention provides examples in which the light source characteristics are manipulated. Examples are the manipulation of the delivery of fast-light pulses and/or the delivery of light at different wavelengths. The present invention also provides examples in which incremental additions of retinal influences the function of the light-driven energy generator. In addition, a proteorhodopsin gene or protein variant can be selected to determine an absorption spectra of the light absorbing pigment to change the kinetics of the light energy generator, for instance to meet a design/functional criteria of an application wherein proteorhodopsin is utilized.

#### BRIEF DESCRIPTION OF THE FIGURES

The objectives and advantages of the present invention will be understood by reading the following detailed description in conjunction with the drawings, in which:

- FIG. 1 illustrates the phylogenetic tree of bacterial 16S rRNA gene sequences including that encoded on the 130 kb bacterioplankton BAC clone (EBAC31A8).
- FIG. 2 provides a nucleotide sequence of polymerase chain reaction primer 1 (Sequence ID No:2) used to amplify a proteorhodopsin gene.
- FIG. 3 provides a nucleotide sequence of polymerase chain reaction primer 2 (Sequence ID No:3) used to amplify a proteorhodopsin gene.
- FIG. 4 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:4) amplified from clone EBAC31A8 (Sequence ID No:1) using PCR primers 1 (Sequence ID No:2) and 2 (Sequence ID No:3), and the deduced amino acid sequence (Sequence ID No:5) of the proteorhodopsin gene Sequence ID No:4 amplified from clone EBAC31A8 (Sequence ID No:1).
- FIG. 5 provides a map of the secondary structure of the proteorhodopsin protein (Sequence ID No:7). Single letter amino acid codes are used (according to J. Sasaki and J.L. Spudich, Biophys. J. 75:2435, 1998). Predicted retinal binding pocket residues are marked in black.
- FIG. 6 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:8) amplified from clone EBAC40E8 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:9) of the proteorhodopsin gene Sequence ID No:8 amplified from clone EBAC40E8.
- FIG. 7 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:10) amplified from clone EBAC41B4 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:11) of the proteorhodopsin gene Sequence ID No:7 amplified from clone EBAC41B4.

- FIG. 8 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:12) amplified from clone EBAC64A5 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:13) of the proteorhodopsin gene Sequence ID No:12 amplified from clone EBAC64A5.
- FIG. 9 provides a variants map of the DNA sequences of the proteorhodopsin gene with Sequence ID No:4, Sequence ID No:8, Sequence ID No:10, and Sequence ID No:12 that were amplified from clone EBAC38A8, EBAC40E8, EBAC41B4 and EBAC64A5 respectively using the proteorhodopsin-specific PCR primer 1 (Sequence ID No:2) and 2 (Sequence ID No:3). Dots represent sequences having identical sequence as those in Sequence ID No:4.
- FIG. 10 provides a variant map of the deduced amino acid sequences encoded by the proteorhodopsin gene with Sequence ID No:4, Sequence ID No:8, Sequence ID No:10, and Sequence ID No:12 that were amplified from respectively EBAC38A8, EBAC40E8, EBAC41B4 and EBAC64A5 using the proteorhodopsin-specific primer 1 (Sequence ID No:2) and 2 (Sequence ID No:3). Lower case represents the PCR primer sequence region. Dots represent residues having identical sequence as those in Sequence ID No:5.
- FIG. 11 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:14) amplified from clone HOT0m1 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:15) of the proteorhodopsin gene Sequence ID No:14 amplified from clone HOT0m1.
- FIG. 12 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:16) amplified from clone HOT75m1 using PCR primers 1 (Sequence ID

- No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:17) of the proteorhodopsin gene Sequence ID No:16 amplified from clone HOT75m1.
- FIG. 13 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:18) amplified from clone HOT75m3 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:19) of the proteorhodopsin gene Sequence ID No:18 amplified from clone HOT75m3.
- FIG. 14 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:20) amplified from clone HOT75m4 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:21) of the proteorhodopsin gene Sequence ID No:20 amplified from clone HOT75m4.
- FIG. 15 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:22) amplified from clone HOT75m8 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:23) of the proteorhodopsin gene Sequence ID No:22 amplified from clone HOT75m8.
- FIG. 16 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:24) amplified from clone MB0m1 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:25) of the proteorhodopsin gene Sequence ID No:24 amplified from clone MB0m1.
- FIG. 17 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:26) amplified from clone MB0m2 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence

- (Sequence ID No:27) of the proteorhodopsin gene Sequence ID No:26 amplified from clone MB0m2.
- FIG. 18 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:28) amplified from clone MB20m2 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:29) of the proteorhodopsin gene Sequence ID No:28 amplified from clone MB20m2.
- FIG. 19 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:30) amplified from clone MB20m5 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:31) of the proteorhodopsin gene Sequence ID No:30 amplified from clone MB20m5.
- FIG. 20 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:32) amplified from clone MB20m12 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:33) of the proteorhodopsin gene Sequence ID No:32 amplified from clone MB20m12.
- FIG. 21 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:34) amplified from clone MB40m1 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:35) of the proteorhodopsin gene Sequence ID No:34 amplified from clone MB40m1.
- FIG. 22 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:36) amplified from clone MB40m5 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence

- (Sequence ID No:37) of the proteorhodopsin gene Sequence ID No:36 amplified from clone MB40m5.
- FIG. 23 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:38) amplified from clone MB40m12 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:39) of the proteorhodopsin gene Sequence ID No:38 amplified from clone MB40m12.
- FIG. 24 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:40) amplified from clone MB100m5 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:41) of the proteorhodopsin gene Sequence ID No:40 amplified from clone MB100m5.
- FIG. 25 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:42) amplified from clone MB100m7 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:43) of the proteorhodopsin gene Sequence ID No:42 amplified from clone MB100m7.
- FIG. 26 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:44) amplified from clone MB100m9 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:45) of the proteorhodopsin gene Sequence ID No:44 amplified from clone MB100m9.
- FIG. 27 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:46) amplified from clone MB100m10 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence

- (Sequence ID No:47) of the proteorhodopsin gene Sequence ID No:46 amplified from clone MB100m10.
- FIG. 28 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:48) amplified from clone PALB1 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:49) of the proteorhodopsin gene Sequence ID No:48 amplified from clone PALB1.
- FIG. 29 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:50) amplified from clone PALB2 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:51) of the proteorhodopsin gene Sequence ID No:50 amplified from clone PALB2.
- FIG. 30 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:52) amplified from clone PALB5 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:53) of the proteorhodopsin gene Sequence ID No:52 amplified from clone PALB5.
- FIG. 31 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:54) amplified from clone PALB7 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:55) of the proteorhodopsin gene Sequence ID No:54 amplified from clone PALB7.
- FIG. 32 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:56) amplified from clone PALB6 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence

- (Sequence ID No:57) of the proteorhodopsin gene Sequence ID No:56 amplified from clone PALB6.
- FIG. 33 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:58) amplified from clone PALB8 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:59) of the proteorhodopsin gene Sequence ID No:58 amplified from clone PALB8.
- FIG. 34 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:60) amplified from clone PALE1 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:61) of the proteorhodopsin gene Sequence ID No:60 amplified from clone PALE1.
- FIG. 35 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:62) amplified from clone PALE6 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:63) of the proteorhodopsin gene Sequence ID No:62 amplified from clone PALE6.
- FIG. 36 provides the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:64) amplified from clone PALE7 using PCR primers 1 (Sequence ID No:2) and 2 (Sequence No:3), and the deduced amino acid sequence (Sequence ID No:65) of the proteorhodopsin gene Sequence ID No:64 amplified from PALE7.
- FIG. 37 illustrates a phylogenetic tree of different proteorhodopsin genes.
- FIG. 38 provides an example of an alignment of proteorhodopsin amino acid sequences.
- FIG. 39 provides a light-driven energy generator that utilizes proteorhodopsin.

- FIG. 40 provides an example of a proteorhodopsin-expressing E. coli cell suspension (+) compared to control cells (-), both with all-trans retinal.
- FIG. 41 provides an example of absorption spectra of retinal-constituted proteorhodopsin in E coli membranes and a negative control.
- FIG. 42 provides an example of a light-driven transport of protons by a proteorhodopsin-expressing E. coli cell suspension.
- FIG. 43 provides an example of a transport of [<sup>3</sup>H]TPP<sup>+</sup> in E. coli right-side-out vesicles containing expressed proteorhodopsin, reconstituted with or without 10 μM retinal in the presence of light or in the dark.
- FIG. 44 provides an example of laser flash-induced absorbance changes in suspensions of *E. coli* membranes containing proteorhodopsin.
- FIG. 45 provides an example of absorption spectra of retinal-constituted proteorhodopsin in *E. coli* membranes.

#### DETAILED DESCRIPTION

Although the following detailed description contains many specifics for the purposes of illustration, anyone of ordinary skill in the art will appreciate that many variations and alterations to the following details are within the scope of the invention. Accordingly, the following preferred embodiment of the invention is set forth without any loss of generality to, and without imposing limitations upon, the claimed invention.

# Proteorhodopsin

The present invention provides rhodopsin-like gene and protein sequences retrieved from naturally occurring members of the domain Bacteria. More specifically, the present invention provides a method for the retrieval and amplification of proteorhodopsin genes from DNA samples of naturally occurring marine bacteria. In accordance with exemplary embodiments of the present invention, DNA samples were obtained from naturally occurring marine bacteria such as bacteria from the SAR86 group. Provided as an exemplary embodiment of the SAR86 group, DNA samples were obtained from a bacterioplankton Bacterial Artificial Chromosome (BAC) clone BAC31A8 (also referred to as EBAC31A08). In general, as will be appreciated by those of ordinary skill in the art, suitable DNA samples can also be obtained from other sources, e.g., from a marine environment or from a recombinant DNA library containing genomic fragments of samples of naturally occurring bacteria.

FIG. 1 shows the phylogenetic tree of bacterial 16S rRNA gene sequences including that encoded on the EBAC31A8. FIG. 1 also shows the relationship of EBAC31A8 to the SAR86 bacteria group as well as to the gamma-proteobacteria group. A subclone shotgun library was constructed from BAC clone 31A8, and subclones were sequenced in both directions on the MegaBACE 1000 capillary array electrophoresis DNA sequencing instrument (Molecular Dynamics, Sunnyvale, CA). Sequence analysis of a 130-kb genomic DNA that encodes the ribosomal RNA operon from BAC31A8, reveals an open reading frame encoding a proteorhodopsin. In an exemplary embodiment, the contiguous sequence was assembled using SEQUENCHER 3.1.1 software (Gene Codes Co., Ann Arbor, MI). Other sequencing techniques can also be used, as will be recognized by those skilled in the art. The sequence of the proteorhodopsin-containing contig has been deposited in GenBank under accession #AF279106 and deposit date October 23<sup>rd</sup>, 2000. Appendix A, hereby incorporated, shows the nucleotide sequence of the BAC clone BAC31A8 (Sequence ID No:1)

which contains the 130 kilobases genomic DNA from a naturally occurring marine bacterium.

Proteorhodopsin was amplified from the 130 kilobase bacterioplankton BAC clone 31A8 (Sequence ID No:1) by polymerase chain reaction (PCR), using the proteorhodopsin-specific primers 5'-aCCATGGgtaaattattactgatattagg-3' (Sequence ID No:2 and shown in FIG. 2) and 5'-agcattagaagattctttaacagc-3' (Sequence ID No:3 and shown in FIG. 3). References for PCR are, for instance, The Polymerase Chain Reaction, Mullis et al., Ed. (Birkhauser, Boston, 1994) and U.S. Patent Nos. 4,683,195 and 4,683,202 to Mullis et al. The proteorhodopsin-specific PCR primers include the addition of 3 nucleotides that encoded one amino acid not found in the native gene sequence of clone BAC31A8 (Sequence ID No:6), in the second amino acid position which is a glycine located on the 2<sup>nd</sup> codon ("GGT"). Therefore, compare the second amino acid position in the Sequence ID No:5 using PCR primers 1 and 2 with the native Sequence ID no:7. This addition of one non-native amino acid created a new restriction endonuclease site (NcoI site) not present in the native sequence. allowed subcloning of the amplified fragment into the Ncol restriction site of an expression vector pBAD TOPO TA Cloning® Kit (Invitrogen, La Jolla, CA). The present invention is not limited to the use of this type of expression vector and other expression vectors could also be used.

FIG. 4 shows the nucleotide sequence of the proteorhodopsin gene (Sequence ID No:4) that results from amplification of the proteorhodopsin-containing DNA in BAC31A8 using proteorhodopsin-specific PCR primers Sequence ID No:2 and Sequence No:3. FIG. 4 also shows the deduced amino acid sequences (Sequence ID No:5) encoded by the proteorhodopsin gene (Sequence ID No:4).

FIG. 5 shows an exemplary embodiment of a secondary structure of proteorhodopsin after it has been folded in a cell membrane 510 and bonded with retinal 520. FIG. 5 shows the native proteorhodopsin gene (Sequence ID No:6) obtained from clone BAC31A8 and encodes a proteorhodopsin protein of 249 amino acids with a molecular weight of 27 kD (Sequence ID No:7). In FIG. 5, 530 indicates seven transmembrane domains, a typical feature of the rhodopsin protein family, that aligned well with the corresponding helices of the archaeal rhodopsins. FIG. 5 also shows the amino acid residues that form a retinal binding pocket indicated by 520. Although the proteorhodopsin proteins shown in FIGS. 4 and 5 both originate from BAC31A8, they differ with respect to the second amino acid position. The reason is that the proteorhodopsin-specific PCR primers that were used to amplify the proteorhodopsin gene from BAC31A8 (which resulted in proteorhodopsin protein as in FIG. 4; Sequence ID No:5) included the addition of 3 nucleotides. These 3 nucleotides encoded one amino acid not found in the native gene sequence (Sequence ID No:6), in the second amino acid position which is a glycine located on the 2<sup>nd</sup> codon ("GGT"). Proteorhodopsin protein (Sequence ID No:7) as shown in FIG. 5 originates from the native gene sequence without the addition of the 3 nucleotides. As mentioned above, the addition of the 3 nucleotides created a new restriction endonuclease site (NcoI site) that was not present in the native sequence and thereby allowed the amplified fragment to be subcloned into the NcoI site of the expression vector.

In the exemplary embodiment presented above, PCR primers with Sequence ID No:2 and Sequence ID No:3 were used. In general, the present invention provides a method for designing different proteorhodopsin-specific PCR primers that are all capable of amplifying a proteorhodopsin gene from DNA samples of naturally occurring microbial populations by polymerase chain reaction. In designing these

primers one first needs to determine a DNA sequence of a proteorhodopsin gene. Then one can design oligodeoxynucleotide primers with a Watson-Crick base pair complementary to 5' and 3' ends of the proteorhodopsin gene.

#### Variants of Proteorhodopsin

In the previous section, an exemplary embodiment is provided of a proteorhodopsin gene and protein. The present invention also provides the retrieval of genetic variations of proteorhodopsin from naturally occurring genetic variations in naturally occurring bacterial populations. These genetic variations in proteorhodopsin sequences result in functional variations in the proteorhodopsin proteins as is discussed below.

The present invention enables one skilled in the art to use the same proteorhodopsin-specific PCR primers as shown in FIGS. 2 and 3 to successfully amplify different sequence variants from DNA originating from mixed naturally occurring bacterial populations when it is compared to for instance the proteorhodopsin gene as shown in FIG. 4. As mentioned above, different proteorhodopsin-specific PCR primers could be used to amplify genetic variants of proteorhodopsin.

FIGS. 6-8 show exemplary embodiments of three different and unique variants of the proteorhodopsin gene that were retrieved from a recombinant DNA library of other naturally occurring bacteria (i.e. the bacterial artificial chromosome library (BAC)). In general, genetic variants could be obtained from different DNA libraries containing naturally occurring bacteria as well as from samples of naturally occurring bacteria.

FIG. 6 shows the variant of the proteorhodopsin gene sequence (Sequence ID No:8) that is amplified from the BAC clone 40 (BAC40E8) with the same proteorhodopsin-

specific PCR primers as provided in Sequence ID No:2 and 3. Accordingly, FIG. 6 also shows the deduced amino acid sequence (Sequence ID No:9) of the genetic variant of proteorhodopsin shown in FIG. 6. FIG. 7 shows the variant of the proteorhodopsin gene sequence (Sequence ID No:10) that is amplified from the BAC clone 41 (BAC41B4) with the same proteorhodopsin-specific PCR primers as provided in Sequence ID No:2 and 3. Accordingly, FIG. 7 also shows the deduced amino acid sequence (Sequence ID No:11) of the genetic variant of proteorhodopsin shown in FIG. 7. FIG. 8 shows the variant of the proteorhodopsin gene sequence (Sequence ID No:12) that is amplified from the BAC clone 64 (BAC64A5) with the same proteorhodopsin-specific PCR primers as provided in Sequence ID No:2 and 3. Accordingly, FIG. 8 also shows the deduced amino acid sequence (Sequence ID No:13) of the genetic variant of proteorhodopsin shown in FIG. 8.

FIG. 9 provides a variants map of the nucleotide sequences of the proteorhodopsin gene Sequence ID No:4, Sequence ID No:8, Sequence ID No:10, and Sequence ID No:12 amplified from respectively BAC31A8, BAC40E8, BAC41B4 and BAC64A5 using the proteorhodopsin-specific PCR primers Sequence ID No:2 and Sequence ID No:3. In FIG. 9 lower case letters represent the PCR primer sequence region. Dots represent residues having identical sequence as those in Sequence ID No:4. These proteorhodopsin gene sequences differ by as much as 31 nucleotides as is shown in FIG. 10. FIG. 10 provides a variant map of the deduced amino acid sequences of the proteorhodopsin genes shown in FIG. 9.

Using the same proteorhodopsin-specific PCR primers, as for instance shown in FIGS. 2 and 3, proteorhodopsin genes were also amplified from bacterioplankton extracts. As mentioned above, any proteorhodopsin-specific PCR primer can be used. These bacterioplankton extracts include those from the Monterey Bay (referred to as MB

clones), the Southern Ocean (Palmer Station, referred to as PAL clones), and waters of the central North Pacific Ocean (Hawaii Ocean Time series station, referred to as HOT clones).

FIGS. 11-36 show exemplary embodiments of different and unique variants of proteorhodopsin that were retrieved from the MB clones, PAL clones, and HOT clones. FIGS. 11-36 each show a variant of a proteorhodopsin gene sequence that is amplified with the same proteorhodopsin-specific PCR primers as provided in Sequence ID No:2 and Sequence ID No:3 from respectively clones HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7. The proteorhodopsin gene sequences retrieved from clones HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7, have respectively Sequence ID Nos: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64. Accordingly, FIGS. 11-36 also show the deduced amino acid sequence of each genetic variant of proteorhodopsin. The deduced amino acid sequence encoded by the proteorhodopsin gene retrieved from clones HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7, have respectively Sequence ID Nos: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, and 65.

In an exemplary embodiment shown in FIG. 37, fifteen different variants of proteorhodopsin in the PCR generated MB gene library 3710 were detected, falling into three clusters. The MB gene library includes MB clones MB0m2, MB40m5, MB20m2, MB40m12, MB100m10, MB20m12, MB40m1, MB100m5, MB20m5, MB100m7, MB0m1, and MB100m9 as well as BAC clones BAC40E8, BAC31A8 and BAC64A5. FIG. 37 is based on a phylogenetic analysis of the inferred amino acids of cloned proteorhodopsin genes. Evolutionary distances calculated from 220 positions were used to infer the tree topology by the neighbor joining method using the PaupSearch program of the Wisconsin Package version 10.0 (Genetics Computer Group (GCG), Madison Wisconsin). Other methods could also be used. The variants of the MB library share at least 97% identity over 248 amino acids, as shown in FIG. 38, and 93% identity at the DNA level. All the PCR amplified proteorhodopsin genes from Antarctic marine bacterioplankton (e.g. the PAL clones) were different from those of Monterey Bay (e.g. the MB clones) sharing 78% identity over 248 amino acids with the Monterey clade. The changes in amino acid sequences were not restricted to the hydrophilic loops, but spread over the entire protein including changes near the retinal binding domain 3830 as shown in FIG. 38, which are predicted retinalbinding residues. FIG. 38 shows an example of a multiple alignment of proteorhodopsin amino acid sequences that were obtained from different clones 3820. The secondary structure is derived from hydropathy plots (boxes 3810 shows transmembrane helices).

## Light-driven energy generator

FIG. 39 provides a light-driven energy generator 3900 that utilizes proteorhodopsin, as obtained from naturally occurring bacteria as described above, to convert light-energy into biochemical energy. Light-driven energy generator 3900 takes advantage of the

functional properties of the proteorhodopsin protein once expressed in, for instance, E. coli and other bacteria. These properties include the ability of proteorhodopsin 3906 to integrate within the cell membrane 3904 of, for instance, E. coli making an integrated proteorhodopsin protein 3908 (also called an integrated cell membrane protein). These properties also include the ability of proteorhodopsin 3906 to bind retinal 3910, making a light absorbing pigment 3912. The source of retinal 3910 is not limited to chromophore retinal but could also include chemical derivatives of retinal, such as 3-methyl-5-(1-pyryl)-2E,4E-pentadienal, 3,7-dimethyl-9-(1-pyryl)-2E,4E,6E,8E-nonatetraenal, all-trans-9-(4-azido-2,3,5,6-tetrafluorophenyl)-3,7dimethyl-2,4,6,8,-nonatetraenal and 2,3-dehydro-4-oxoretinal. Illuminating light absorbing pigment 3912 with a light source 3914 results in a chemiosmotic gradient or proton pump in which light energy 3916 is converted into biochemical energy 3918. The chemiosmotic gradient involves pumping of protons from the inside to the outside of cell membrane 3904. When the protons return to the inside of cell membrane 3904 it produces biochemical energy 3918 via a proton translocating ATP-ase. Finally, the biochemical energy 3918 is harnessed by a mediator 3920 to produce energy 3922 for a particular process. For example, since proteorhodopsin functions as a light driven proton pump, it generates energy in the form of a proton motive force across the host cell membrane upon illumination. This light-driven proton motive force can be converted to many other forms of energy, one example above being the regeneration of adenosine triphosphate (ATP), via a proton-translocating ATPase. This coupling of the proton motive force generated by proteorhodopsin, for use by proton-translocating ATPases to synthesize ATP, could be accomplished both in living cells, as well as in artificially constructed membrane systems such as liposomes. Proteorhodopsin-based systems can convert light energy to a wide variety of useful mechanical, chemical, and electrical energy forms, for many industrial and technological applications. These

include, but are not limited to, use in targeted drug delivery, uses as primary or secondary energy generators for biocatalyic reactors, fuel cells and nano-machines (including molecular motors), as well as uses in molecular switching or data storage devices.

Applications that can potentially benefit from proteorhodopsin-light driven energy generation are, for instance, bio-electronics applications that are aimed to interface, integrate, or substitute the silicon based microelectronics systems as well as molecular devices. Other applications that can potentially benefit from proteorhodopsin-light driven energy generation are, for instance, in bio-materials, wherein proteorhodopsin is integrated as a bio-material in, for instance, optical films for light mediated computer memory applications, optical information storage and pattern recognition.

Alternatively, proteorhodopsin is useful for a process to enhance yield or increase the potential of recombinant protein production or converting the light induced membrane potential into cellular signals, including modulation of gene expression. The biochemical energy derived from functional proteorhodopsin exposed to light could be harnessed to support a variety of cellular processes. For instance, the energy derived from light-mediated proton pumping could be used to enhance the production of secondary metabolites, or recombinant proteins in host cells, such as *E. coli*. Often, production of specific compounds in the biotechnology industry is limited, since their optimal expression or production occurs in the late stationary phase of growth, when energy reserves of the host cells are low. Retinal-bound proteorhodopsin expressed in such cells would provide an ample source of biochemical energy, by simple illumination. Proteorhodopsin-mediated light driven proton production could enhance any variety of biosynthetic or physiological processes which require energy.

The biochemical energy derived from proteorhodopsin light driven proton pumping could also be converted to other generally useful energy forms, for example electricity. Microbial fuel cells currently use carbon-based compounds, such as glucose, as the primary energy source. Via specific mediators of reduction potential (e.g. electrons), these microbial fuel cells convert cellular biochemical energy to electrical potential. Unlike carbon-based microbial fuel cells, proteorhodopsin uses light as the energy source, that can then be converted into a chemiosmotic potential, and finally into cellular biochemical energy by membrane-bound proton ATP-ases. Therefore, the use of proteorhodopsin could be employed to derive energy from light as the primary or supplementary energy source, that could then be converted into electrical potential (analogous microbial fuel cells that derive their energy from glucose).

In addition to energy generation in vivo in living cells, membranes containing proteorhodopsin could be used to enhance or enable other specific processes in vitro. Polymers produced from proteorhodopsin-containing membranes may have specific properties that could be used similarly to those containing bacteriorhodopsin. One example includes the use of these light sensitive molecules for optical computing applications.

As shown in FIG. 39, the kinetics of proteorhodopsin as it is utilized in 3900 is influenced by various factors such as the type of light source 3914 and the manipulation of light source 3914 in terms of frequency and/or wavelength at which the light 3916 is delivered. Light source 3914 could be any type of light source that delivers light energy 3916 that would be absorbed by light absorbing pigment 3918.

For example, the light source 3914 could be tuned to optimally excite rhodopsin variances with an absorbance maximum of 490 nm or alternatively those rhodopsins with an absorbance maximum of 520 nm. Manipulation of the light source 3914 or the light 3916 being emitted by the light source 3914, for example, involves changing the frequency of fast-light pulses or the delivery of light 3916 as individual pulses, a train of pulses, or a continuous source of light. Manipulation also involves changing the wavelength of the delivery of light 3916 at different wavelengths. In addition, as is clear for one skilled in the art, changing the frequency and/or amount of retinal that will bind within integrated cell membrane protein 3908 also varies the function of proteorhodopsin. Finally, as was mentioned in the previous section, genetic variants of proteorhodopsin result in variants of the proteorhodopsin proteins that changes the kinetics of 3600 due to a difference in absorption of light at different wavelengths. The functional expression of such variation in these proteorhodopsin proteins adds another source of variation to the kinetics of proteorhodopsin as it is utilized in 3900.

As shown in FIG. 39, the light-driven energy generator includes a host 3902. In the present invention, as a preferred embodiment, host 3902 is a cell membrane preparation of *E. coli*. However, the present invention is not limited to the use of *E. coli* and, alternatively, other bacteria or eukaryotes could be used to provide host 3902 as an intact cell (in vivo) and/or as a cell membrane preparation (in vitro). For example, but not limited to, bacteria and yeast with developed genetic systems such as Bacillus spp. Species, Saccharomyces spp., Streptomyces spp. or Pichia spp. could be used as host for the expression of proteorhodopsin. In addition, in case a cell membrane preparation (in vitro) is used, host 3902 becomes equivalent to cell membrane 3904.

The light-driven energy generator 3900, as shown in FIG. 39, further includes proteorhodopsin 3906. Proteorhodopsin is presented in the form of the earlier presented expression vector containing a proteorhodopsin gene or one of its variants. Once proteorhodopsin 3906 has been put into host 3902, the proteorhodopsin expression vector expresses the proteorhodopsin protein in host 3902. An integral cell membrane protein 3908 is created in which the proteorhodopsin protein inserts into and folds properly within the cell membrane 3904. This is accomplished in the *E. coli* host by virtue of the native signal sequence found in the 5' end of the proteorhodopsin gene. It could also be accomplished by replacement of native sequence with another host-specific signal sequence in non-*E. coli* host systems.

As shown in FIG. 39, once retinal 3910 is added to cell membrane 3904, retinal 3910 binds within integrated cell membrane protein 3908 and forms a light absorbing pigment **3912**. The particular example of FIG. 40 shows an integrated proteorhodopsin protein 3908 bound to retinal 3910 in E. coli. Chemical derivatives of retinal (as discussed above) could also be used as a substitute chromophore to generate functional proteorhodopsin. For the particular example of FIG. 40, the proteorhodopsin protein was cloned with its native signal sequence and included an addition of the V5 epitope, and a polyhistidine tail in the C-terminus. proteorhodopsin protein was expressed in host 3902, i.e. E. coli outer-membrane protease-deficient strain UT5600, and induced with 0.2 % arabinose for 3 hours. Cell membranes 3904 were prepared and resuspended in 50 mM Tris-Cl (pH 8.0) and 5 mM MgCl<sub>2</sub>. **FIG. 40** shows a proteorhodopsin-expressing *E.coli* cell suspension. After 3 hours of induction in the presence of 10 µM all-trans retinal, cells expressing the protein acquire a reddish pigmentation as indicated by 4010 and the + (plus) symbol. FIG. 40 also shows that a cell suspension using the same PCR primers

(Sequence ID No:2 and 3) but now in opposite orientation as a negative control, did not acquire a reddish pigmentation as indicated by 4020 and the – (minus) symbol.

FIG. 41 shows an exemplary embodiment of the absorption spectra of light absorbing pigment 3912 upon illumination with light source 3914 as is shown in FIG 39. As mentioned above, the light absorbing pigment is a retinal-reconstituted proteorhodopsin in E. coli. FIG. 41 shows absorption spectra of light absorbing pigment 3912 as well as a negative control. After retinal 3910 addition to integrated proteorhodopsin protein 3908, light absorbing pigment 3912 was made. The retinal 3910 addition was done at selected time points, i.e. 10, 20, 30 and 40 min, and shows a progression from low to high absorption values indicated by respectively 4110, 4120, 4130 and 4140 upon illumination with light source 3914. FIG. 41 also shows the absorption spectra of retinal 3910 addition at these similar time points but now to a negative control of retinal 3910 containing a proteorhodopsin 3906 that was created using the same PCR primers in opposite orientation. 4150, 4160, 4170 and 4180 indicate the four absorption spectra for the negative control. An absorption peak at 520 nm was observed after 10 minutes (4110) of incubation as illustrated in FIG. 41. On further addition of retinal, the peak at 520 nm increased, and had a ~100 nm half bandwidth. The 520 nm absorption peak was generated only in membranes containing proteorhodopsin 3906, and only in the presence of retinal 3910. The red shifted  $\lambda$ max of retinal ( $\lambda$ max = 370 nm in the free state) is indicative of a protonated Shiff base covalent linkage of retinal to proteorhodopsin.

FIG. 42 shows an exemplary embodiment of the light mediated proton pump of the light-driven energy generator 3900 indicating the conversion of light energy 3916 as shown in FIG. 39. The proton pump action is illustrated by measuring pH changes in

the medium surrounding the host 3902, which in this particular example involves a cell suspension of  $E.\ coli$ , illuminated by light source 3914. The beginning and cessation of illumination (with yellow light >485 nm delivered by 3916) is indicated 4110 ("ON") and 4120 ("OFF") respectively. The cells were suspended in 10 mM NaCl, 10 mM MgSO<sub>4</sub>·7H<sub>2</sub>O and 100  $\mu$ M CaCl<sub>2</sub>. Net outward transport of protons was observed solely in proteorhodopsin-containing  $E.\ coli$  cells, and only in the presence of retinal 3910 and light 3916 and is indicated by 4210 in FIG. 42. Light-induced acidification of the medium was completely abolished by the presence of 10  $\mu$ M of the protonophore CCCP.

FIG. 43 is an exemplary embodiment showing that illumination by light source 3914 generates an electrical potential at the membrane 3904 in proteorhodopsin-containing right-side-out membrane vesicles, in the presence of retinal 3910, reaching –90 mV after 2 minutes from light 3916 onset. Transport of [³H]TPP+ in *E. coli* right-side-out vesicles containing expressed proteorhodopsin, reconstituted with (4310 and 4320) or without (4330 and 4340) 10 μM retinal 3910 in the presence of light (4310 and 4330) delivered by the light source 3914 or in the dark (4320 and 4340). FIG. 43 shows that proteorhodopsin, in its form of 3912 as a light absorbing pigment, pumps protons from the inside to the outside of cell membrane in a physiologically relevant range. The ability of proteorhodopsin to generate a physiologically significant membrane potential, even when heterologously expressed in nonnative membranes, is consistent with the proton pumping function for proteorhodopsin in the native gamma proteobacteria from which it is derived.

FIG. 44 is an exemplary embodiment showing that proteorhodopsin can have a fast photocycle and can therefore be characterized as a fast and therefore efficient

transporter of protons. For the particular example of FIG. 44, light absorbing pigment 3912 is induced by laser pulses delivered by light source 3914. Laser pulse-induced absorption changes are shown by 3912 in host 3902, which in this case are suspensions of E. coli membranes containing proteorhodopsin. A 532-nm pulse (6 ns duration, 40 mJ) was delivered at time 0 and absorption changes were monitored at various wavelengths in the visible range in a lab-constructed pulse photolysis system. 64 transients were collected for each wavelength. 4410 indicates transients at 3 wavelengths exhibiting maximal amplitudes. 4420 indicates absorption difference absorption spectra calculated from amplitudes at 0.5 ms (indicated by 4430) and between 0.5 ms and 5.0 ms (indicated by 4440). In 4410, transient depletion occurred near the absorption maximum of pigment 3912 (500-nm trace indicated by 4450), and transient absorption increase was detected at 400 nm (indicated by 4460) and 590 nm (indicated by 4470), indicating a functional photocyclic reaction pathway. In 4420, the absorption difference spectrum shows that within 0.5 ms an intermediate with maximal absorption near 400 nm is produced (indicated by 4430), typical of unprotonated Schiff base forms (M intermediates) of retinylidene pigments. The 5-ms minus 0.5-ms difference spectrum 4440 shows that following M decay an intermediate species redshifted from the unphotolyzed 520-nm state appears. The decay of proteorhodopsin final intermediate is the rate limiting step in the photocycle and is fit well by a single exponential process of 15 ms, with an upward baseline shift of 13% of the initial amplitude.

As mentioned above, a proteorhodopsin gene or protein variant can be selected to determine an absorption spectra of the light absorbing pigment to change the kinetics of the light energy generator 3900, for instance to meet a design/functional criteria of an application wherein proteorhodopsin is utilized. FIG. 45 shows an exemplary

embodiment of different absorption spectra of retinal-reconstituted proteorhodopsins in *E. coli* as a function of wavelength 4510. As shown in FIG. 45, the absorbance 4520 is different and depends on the clone from which the proteorhodopsin was amplified. In this particular example, 5 µm all-trans retinal was added to the membranes suspensions in a 100 mM phosphate buffer, with a pH 7.0, and absorption spectra were recorded. The four spectra 4530, 4540, 4550, and 4560 are respectively for the proteorhodopsin genes retrieved from clones HOT75m4, PALE6, HOT0m1, and BAC31A8 at 1 hour after retinal addition. The proteorhodopsin gene retrieved from clone HOT75m4 4530 and PALE6 4540 produced a blue (490 nm) absorption maximum. The proteorhodopsin gene retrieved from clone HOT0m1 4550 and BAC31A8 4560 produced a green (527 nm) absorption maximum. In general, a range of wavelengths could be obtained that is not limited to the range shown in the example of FIG. 45.

It will be clear to one skilled in the art that the above embodiment may be altered in many ways without departing from the scope of the invention, such as for instance by mutagenesis to change the genetic sequence of proteorhodopsin and thereby changing the kinetics of the proteorhodopsin protein once it is expressed. Accordingly, the following claims and their legal equivalents should determine the scope of the invention.

# **DEPOSITS**

Depository address: 10801 University Boulevard, Manassas, VA 20110, USA.

The Escherichia coli containing cloned DNA BAC 31A8 having assigned ATCC number PTA-3083, the Escherichia coli containing cloned DNA BAC 40E8 having assigned ATCC number PTA-3082, the Escherichia coli containing cloned DNA BAC 41B4 having assigned ATCC number PTA-3080, and the Escherichia coli containing cloned DNA BAC 64A5 having assigned ATCC number PTA-3081, all having been deposited on February 21, 2001 with the ATCC Patent Depository.

The Escherichia coli containing a plasmid PAL E6 having assigned ATCC number PTA-3250, the Escherichia coli containing a plasmid HOT 0m1 having assigned ATCC number PTA-3251, the Escherichia coli containing a plasmid HOT 75m4 having assigned ATCC number PTA-3252, and the Escherichia coli containing cloned DNA BAC64A5 having assigned ATCC number PTA 3082, all having been deposited on March 30, 2001 with the ATCC Patent Depository.

# LIGHT-DRIVEN ENERGY GENERATION USING PROTEORHODOPSIN

# LIST OF SEQUENCES THAT ARE LISTED IN THE INCORPORATED SEQUENCE LISTING

Sequence ID No:1 bacterial artificial chromosome (BAC) clone 31A8 (EBAC31A8).

Sequence ID No:2 nucleotide sequence of proteorhodopsin-specific polymerase chain reaction (PCR) primer 1.

Sequence ID No:3 nucleotide sequence of proteorhodopsin-specific polymerase chain reaction (PCR) primer 2.

Sequence ID No:4 nucleotide sequence of the proteorhodopsin gene amplified from clone EBAC31A8 (Sequence ID No. 1) using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:5 deduced amino acid sequences of the proteorhodopsin gene amplified from clone EBAC31A8 (Sequence ID NO:4).

Sequence ID No:6 native proteorhodopsin nucleotide sequence from clone EBAC31A8 (Sequence ID No:1).

Sequence ID No:7 deduced amino acid sequences of the native proteorhodopsin nucleotide sequence from clone EBAC31A8 (Sequence ID No:6).

Sequence ID No:8 nucleotide sequence of the proteorhodopsin gene amplified from clone EBAC40E8 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:9 deduced amino acid sequences of the proteorhodopsin gene amplified from clone EBAC40E8 (Sequence ID NO:8).

Sequence ID No:10 nucleotide sequence of the proteorhodopsin gene amplified from clone EBAC41B4 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:11 deduced amino acid sequences of the proteorhodopsin gene amplified from clone EBAC41B4 (Sequence ID NO:10).

Sequence ID No:12 nucleotide sequence of the proteorhodopsin gene amplified from clone EBAC64A5 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:13 deduced amino acid sequences of the proteorhodopsin gene amplified from clone EBAC64A5 (Sequence ID NO:12).

Sequence ID No:14 nucleotide sequence of the proteorhodopsin gene amplified from clone HOT0m1 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:15 deduced amino acid sequences of the proteorhodopsin gene amplified from clone HOT0m1 (Sequence ID NO:14).

Sequence ID No:16 nucleotide sequence of the proteorhodopsin gene amplified from clone HOT75m1 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:17 deduced amino acid sequences of the proteorhodopsin gene amplified from clone HOT75m1 (Sequence ID NO:16).

Sequence ID No:18 nucleotide sequence of the proteorhodopsin gene amplified from clone HOT75m3 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:19 deduced amino acid sequences of the proteorhodopsin gene amplified from clone HOT75m3 (Sequence ID NO:18).

Sequence ID No:20 nucleotide sequence of the proteorhodopsin gene amplified from clone HOT75m4 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:21 deduced amino acid sequences of the proteorhodopsin gene amplified from clone HOT75m4 (Sequence ID NO:20).

Sequence ID No:22 nucleotide sequence of the proteorhodopsin gene amplified from clone HOT75m8 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:23 deduced amino acid sequences of the proteorhodopsin gene amplified from clone HOT75m8 (Sequence ID NO:22).

Sequence ID No:24 nucleotide sequence of the proteorhodopsin gene amplified from clone MB0m1 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:25 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB0m1 (Sequence ID NO:24).

Sequence ID No:26 nucleotide sequence of the proteorhodopsin gene amplified from clone MB0m2 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:27 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB0m2 (Sequence ID NO:26).

Sequence ID No:28 nucleotide sequence of the proteorhodopsin gene amplified from clone MB20m2 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:29 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB20m2 (Sequence ID NO:28).

Sequence ID No:30 nucleotide sequence of the proteorhodopsin gene amplified from clone MB20m5 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:31 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB20m5 (Sequence ID NO:30).

Sequence ID No:32 nucleotide sequence of the proteorhodopsin gene amplified from clone MB20m12 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:33 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB20m12 (Sequence ID NO:32).

Sequence ID No:34 nucleotide sequence of the proteorhodopsin gene amplified from clone MB40m1 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:35 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB40m1 (Sequence ID NO:34).

Sequence ID No:36 nucleotide sequence of the proteorhodopsin gene amplified from clone MB40m5 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:37 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB40m5 (Sequence ID NO:36).

Sequence ID No:38 nucleotide sequence of the proteorhodopsin gene amplified from clone MB40m12 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:39 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB40m12 (Sequence ID NO:38).

Sequence ID No:40 nucleotide sequence of the proteorhodopsin gene amplified from clone MB100m5 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:41 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB100m5 (Sequence ID NO:40).

Sequence ID No:42 nucleotide sequence of the proteorhodopsin gene amplified from clone MB100m7 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:43 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB100m7 (Sequence ID NO:42).

Sequence ID No:44 nucleotide sequence of the proteorhodopsin gene amplified from clone MB100m9 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:45 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB100m9 (Sequence ID NO:44).

Sequence ID No:46 nucleotide sequence of the proteorhodopsin gene amplified from clone MB100m10 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:47 deduced amino acid sequences of the proteorhodopsin gene amplified from clone MB100m10 (Sequence ID NO:46).

Sequence ID No:48 nucleotide sequence of the proteorhodopsin gene amplified from clone PALB1 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:49 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALB1 (Sequence ID NO:48).

Sequence ID No:50 nucleotide sequence of the proteorhodopsin gene amplified from clone PALB2 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:51 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALB2 (Sequence ID NO:50).

Sequence ID No:52 nucleotide sequence of the proteorhodopsin gene amplified from clone PALB5 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:53 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALB5 (Sequence ID NO:52).

Sequence ID No:54 nucleotide sequence of the proteorhodopsin gene amplified from clone PALB7 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:55 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALB7 (Sequence ID NO:54).

Sequence ID No:56 nucleotide sequence of the proteorhodopsin gene amplified from clone PALB6 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:57 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALB6 (Sequence ID NO:56).

Sequence ID No:58 nucleotide sequence of the proteorhodopsin gene amplified from clone PALB8 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:59 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALB8 (Sequence ID NO:58).

Sequence ID No:60 nucleotide sequence of the proteorhodopsin gene amplified from clone PALE1 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:61 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALE1 (Sequence ID NO:60).

Sequence ID No:62 nucleotide sequence of the proteorhodopsin gene amplified from clone PALE6 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:63 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALE6 (Sequence ID NO:62).

Sequence ID No:64 nucleotide sequence of the proteorhodopsin gene amplified from clone PALE7 using PCR primers according to Sequence ID No:2 and Sequence No:3.

Sequence ID No:65 deduced amino acid sequences of the proteorhodopsin gene amplified from clone PALE7 (Sequence ID NO:64).

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09	ıta ctaatatata attagtttat	agtaatggct attgctccaa taacttaata	ttgttatatc agtaatggct

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0	900	tagaggcaga	ataaactcgt	aactatacat	ttgactgatc	ttaaccatct	tcccaaaaga

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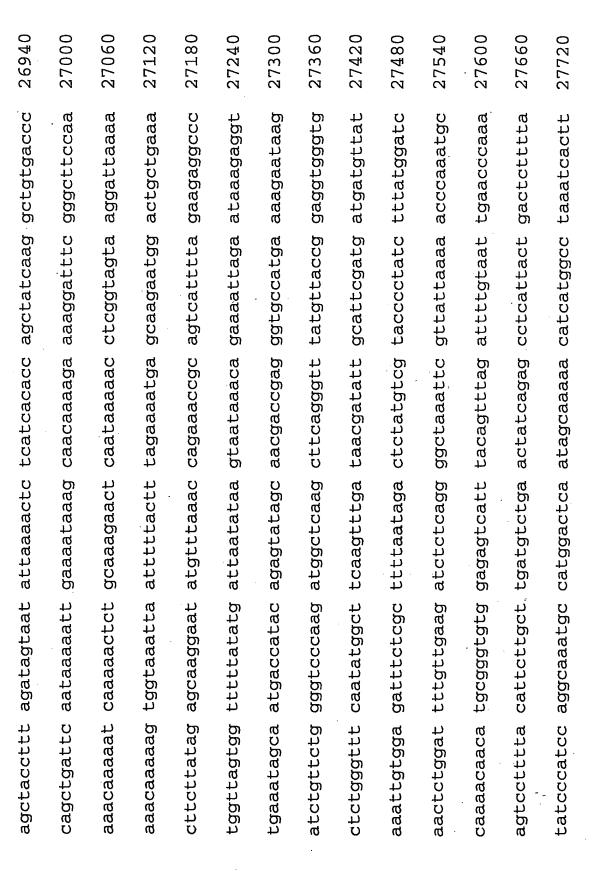
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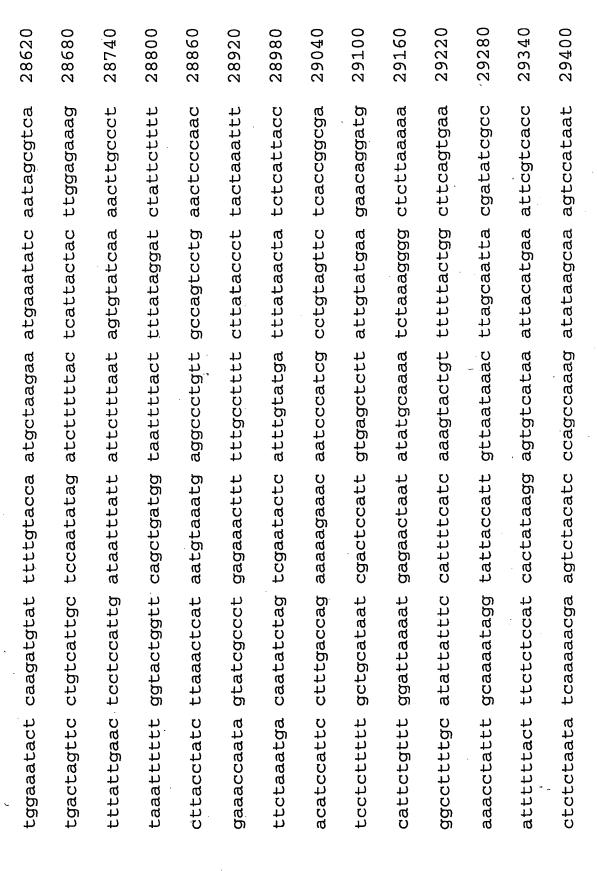
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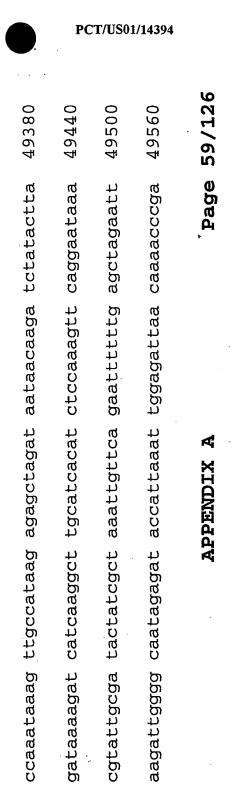
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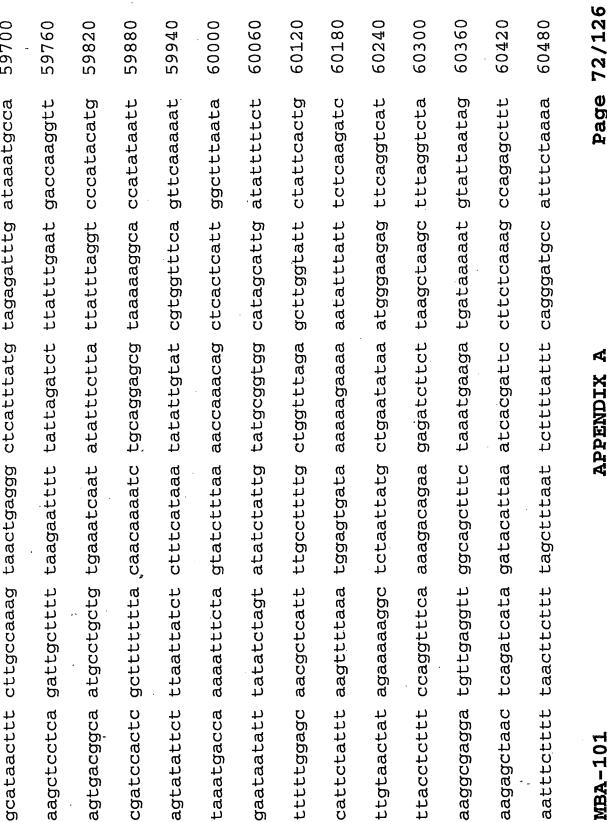
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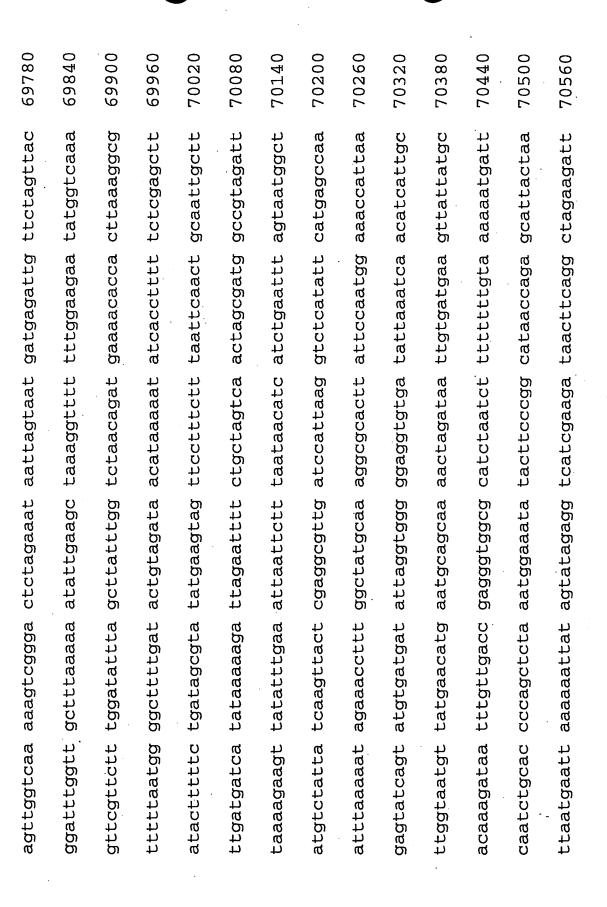
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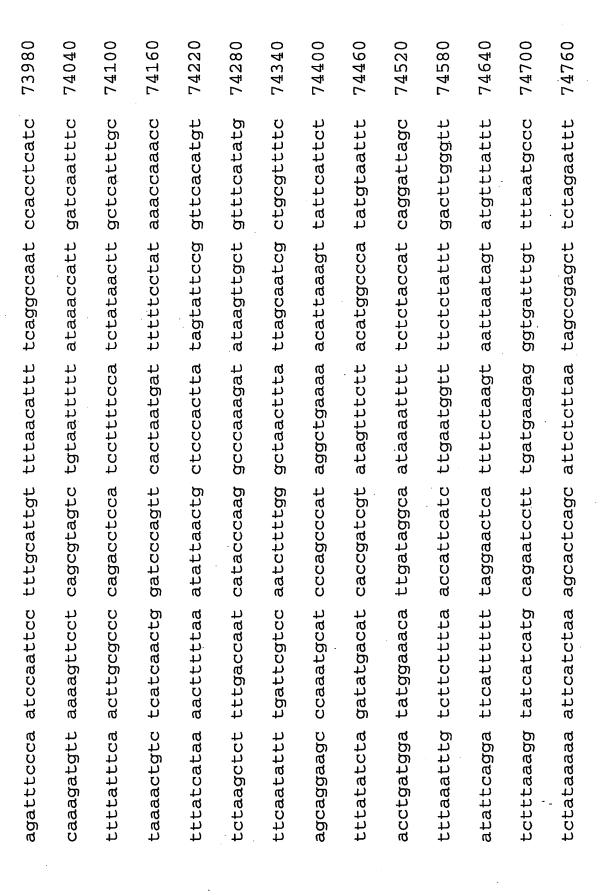
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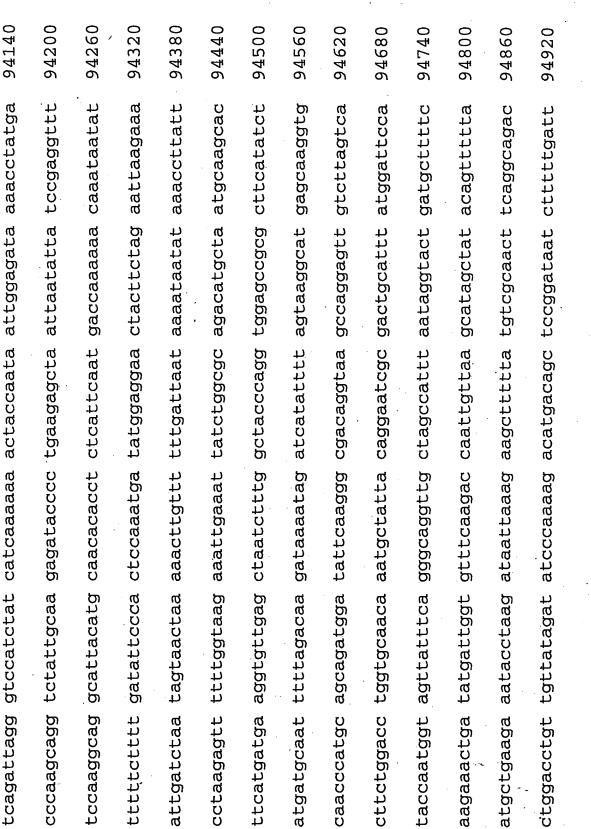
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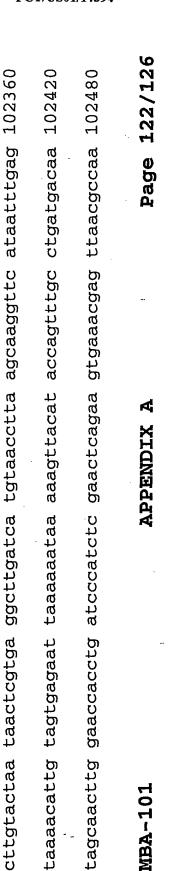
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APPENDIX

104700 104220 104340 104400 104520 104580 104640 104760 104940 104280 104460 104820 104880 105000 ctattcctct ttcttcttga ttttgttatt gaatgggaga attctgttgt ggatgatgat caccttcact agaattttga ttttattatt tatccgtccc tggcaattat ttcctgtcat aagtagccct ctagtgtttg ttacctgcat tgaggtattt ctgaagattc aaccgaaact gttccaacaa gcctggtgaa tgcgcatttt agcctttcaa aaagataaga tgattatcta tctataactt ccaagaacta ttatatttcg acatttgttg gtatctgctg gtggtggatg aattctttt cccaccagat tttttttccg cattccaggt gcttggcatt taaaatttga aattagagct ccccttaac agccatagga gcattccagt tactgcttca gttttggaat tcccatgccc atcgtcttct caatttcttc caatagttac gtatatgaag tttggtcttc gatcagctct gactaaactc ttatgatata tgcctgatct cattttcttt tatgatcatc gtctcaagag atctaaaaaa gatacttctt tggatagcaa gcatcaaagt tctatatcgc atattcgtag cttaattgtt aagtcattaa tttttaaata ttagcttcaa tcaccttgtc tctgttccaa ttttcgtgat ccatagattt gaaataaaaa ctctgcttct agactctctt ataacctgga ttcatgcctt ttctttccac ccttcctgcc taaatatgct attttcctt tgaaagcatt tcttccatcc aagaaatact gacatttagg cccagaaagt

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105060 105184 105120 105180 cattettett eegggeattg ttetacetgt atgeteatea ateaaaagaa eeteaeegtt aaatttcata atttttaaat tagaaacaga gtaagcccat ctgaggctcc aagccgattc ttgcttgaac gctctaagtg taagaagctt taatccacat tctttttaaa cctaaccaaa cagc

## **CLAIMS**

## What is claimed is:

- 1. A proteorhodopsin gene, comprising an isolated DNA sequence for encoding a proteorhodopsin protein.
  - 2. The proteorhodopsin gene of claim 1, wherein said proteorhodopsin gene is retrieved from a genomic fragment of a sample of naturally occurring bacteria.
    - 3. The proteorhodopsin gene of claim 2, wherein said naturally occurring bacteria are marine proteobacteria.
    - 4. The proteorhodopsin gene of claim 2, wherein said naturally occurring bacteria are SAR86 bacteria.
    - The proteorhodopsin gene of claim 2, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.
      - 6. The proteorhodopsin gene of claim 5, wherein said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.

- 7: The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC31A8, said proteorhodopsin gene is Sequence ID No:4 and said proteorhodopsin protein is Sequence ID No:5.
- 8. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC40E8, said proteorhodopsin gene is Sequence ID No:8 and said proteorhodopsin protein is Sequence ID No:9.
- 9. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC41B4, said proteorhodopsin gene is Sequence ID No:10 and said proteorhodopsin protein is Sequence ID No:11.
- 10. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC64A5, said proteorhodopsin gene is Sequence ID No:12 and said proteorhodopsin protein is Sequence ID No:13.
- 11. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT0m1, said proteorhodopsin gene is Sequence ID No:14 and said proteorhodopsin protein is Sequence ID No:15.

- 12. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m1, said proteorhodopsin gene is Sequence ID No:16 and said proteorhodopsin protein is Sequence ID No:17.
- 13. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m3, said proteorhodopsin gene is Sequence ID No:18 and said proteorhodopsin protein is Sequence ID No:19.
- 14. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m4, said proteorhodopsin gene is Sequence ID No:20 and said proteorhodopsin protein is Sequence ID No:21.
- 15. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m8, said proteorhodopsin gene is Sequence ID No:22 and said proteorhodopsin protein is Sequence ID No:23.
- 16. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB0m1, said proteorhodopsin gene is Sequence ID No:24 and said proteorhodopsin protein is Sequence ID No:25.

- 17. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB0m2, said proteorhodopsin gene is Sequence ID No:26 and said proteorhodopsin protein is Sequence ID No:27.
- 18. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB20m2, said proteorhodopsin gene is Sequence ID No:28 and said proteorhodopsin protein is Sequence ID No:29.
- 19. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB20m5, said proteorhodopsin gene is Sequence ID No:30 and said proteorhodopsin protein is Sequence ID No:31.
- 20. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB20m12, said proteorhodopsin gene is Sequence ID No:32 and said proteorhodopsin protein is Sequence ID No:33.
- 21. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB40m1, said proteorhodopsin gene is Sequence ID No:34 and said proteorhodopsin protein is Sequence ID No:35.

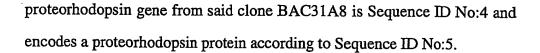
- 22. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB40m5, said proteorhodopsin gene is Sequence ID No:36 and said proteorhodopsin protein is Sequence ID No:37.
- 23. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB40m12, said proteorhodopsin gene is Sequence ID No:38 and said proteorhodopsin protein is Sequence ID No:39.
- 24. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m5, said proteorhodopsin gene is Sequence ID No:40 and said proteorhodopsin protein is Sequence ID No:41.
- 25. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m7, said proteorhodopsin gene is Sequence ID No:42 and said proteorhodopsin protein is Sequence ID No:43.
- 26. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m9, said proteorhodopsin gene is Sequence ID No:44 and said proteorhodopsin protein is Sequence ID No:45.

- 27. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m10, said proteorhodopsin gene is Sequence ID No:46 and said proteorhodopsin protein is Sequence ID No:47.
- 28. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB1, said proteorhodopsin gene is Sequence ID No:48 and said proteorhodopsin protein is Sequence ID No:49.
- 29. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB2, said proteorhodopsin gene is Sequence ID No:50 and said proteorhodopsin protein is Sequence ID No:51.
- 30. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB5, said proteorhodopsin gene is Sequence ID No:52 and said proteorhodopsin protein is Sequence ID No:53.
- 31. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB7, said proteorhodopsin gene is Sequence ID No:54 and said proteorhodopsin protein is Sequence ID No:55.

- 32. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB6, said proteorhodopsin gene is Sequence ID No:56 and said proteorhodopsin protein is Sequence ID No:57.
- 33. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB8, said proteorhodopsin gene is Sequence ID No:58 and said proteorhodopsin protein is Sequence ID No:59.
- 34. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALE1, said proteorhodopsin gene is Sequence ID No:60 and said proteorhodopsin protein is Sequence ID No:61.
- 35. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALE6, said proteorhodopsin gene is Sequence ID No:62 and said proteorhodopsin protein is Sequence ID No:63.
- 36. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALE7, said proteorhodopsin gene is Sequence ID No:64 and said proteorhodopsin protein is Sequence ID No:65.

- 37. The proteorhodopsin gene of claim 1, wherein said proteorhodopsin gene is amplified from a genomic fragment by polymerase chain reaction.
  - 38. The proteorhodopsin gene of claim 37, wherein said polymerase chain reaction is performed by primers with Sequence ID No:2 and Sequence ID No:3.
- 39. The proteorhodopsin gene of claim 1, wherein said proteorhodopsin gene is derived from a marine environment and placed in an expression vector for producing said proteorhodopsin protein in a host.
  - 40. The proteorhodopsin gene of claim 39, wherein said host is an artificial membrane system.
  - 41. The proteorhodopsin gene of claim 39, wherein said host is a bacterium.
    - 42. The proteorhodopsin gene of claim 41, wherein said host is a cell membrane preparation of said bacterium.
  - 43. The proteorhodopsin gene of claim 39, wherein said host is an eukaryote.
    - 44. The proteorhodopsin gene of claim 43, wherein said host is a cell membrane preparation of said eukaryote.

- 45. A method of retrieving a proteorhodopsin gene, comprising the steps of:
  - (a) providing a sample of naturally occurring bacteria;
  - (b) extracting a genomic fragment of said sample of naturally occurring bacteria; and
  - (c) amplifying said proteorhodopsin gene from said genomic fragment using polymerase chain reaction.
  - 46. The method of claim 45, further comprising the step of creating an expression vector containing said proteorhodopsin gene.
  - 47. The method of claim 45, wherein said naturally occurring bacteria are marine proteobacteria.
  - 48. The method of claim 45, wherein said naturally occurring bacteria are SAR86 bacteria.
  - 49. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.
    - 50. The method of claim 49, said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.
  - 51. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC31A8, and wherein said amplified



- 52. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC40E8, and wherein said amplified proteorhodopsin gene from said clone BAC40E8 is Sequence ID No:8 and encodes a proteorhodopsin protein according to Sequence ID No:9.
- 53. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC41B4, and wherein said amplified proteorhodopsin gene from said clone BAC41B4 is Sequence ID No:10 and encodes a proteorhodopsin protein according to Sequence ID No:11.
- 54. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC64A5, and wherein said amplified proteorhodopsin gene from said clone BAC64A5 is Sequence ID No:12 and encodes a proteorhodopsin protein according to Sequence ID No:13.
- 55. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT0m1, and wherein said amplified proteorhodopsin gene from said clone HOT0m1 is Sequence ID No:14 and encodes a proteorhodopsin protein according to Sequence ID No:15.
- 56. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m1, and wherein said amplified

proteorhodopsin gene from said clone HOT75m1 is Sequence ID No:16 and encodes a proteorhodopsin protein according to Sequence ID No:17.

- 57. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m3, and wherein said amplified proteorhodopsin gene from said clone HOT75m3 is Sequence ID No:18 and encodes a proteorhodopsin protein according to Sequence ID No:19.
- 58. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m4, and wherein said amplified proteorhodopsin gene from said clone HOT75m4 is Sequence ID No:20 and encodes a proteorhodopsin protein according to Sequence ID No:21.
- 59. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m8, and wherein said amplified proteorhodopsin gene from said clone HOT75m8 is Sequence ID No:22 and encodes a proteorhodopsin protein according to Sequence ID No:23.
- 60. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB0m1, and wherein said amplified proteorhodopsin gene from said clone MB0m1 is Sequence ID No:24 and encodes a proteorhodopsin protein according to Sequence ID No:25.
- 61. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB0m2, and wherein said amplified

proteorhodopsin gene from said clone MB0m2 is Sequence ID No:26 and encodes a proteorhodopsin protein according to Sequence ID No:27.

- 62. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB20m2, and wherein said amplified proteorhodopsin gene from said clone MB20m2 is Sequence ID No:28 and encodes a proteorhodopsin protein according to Sequence ID No:29.
- 63. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB20m5, and wherein said amplified proteorhodopsin gene from said clone MB20m5 is Sequence ID No:30 and encodes a proteorhodopsin protein according to Sequence ID No:31.
- 64. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB20m12, and wherein said amplified proteorhodopsin gene from said clone MB20m12 is Sequence ID No:32 and encodes a proteorhodopsin protein according to Sequence ID No:33.
- 65. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB40m1, and wherein said amplified proteorhodopsin gene from said clone MB40m1 is Sequence ID No:34 and encodes a proteorhodopsin protein according to Sequence ID No:35.
- 66. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB40m5, and wherein said amplified

proteorhodopsin gene from said clone MB40m5 is Sequence ID No:36 and encodes a proteorhodopsin protein according to Sequence ID No:37.

- 67. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB40m12, and wherein said amplified proteorhodopsin gene from said clone MB40m12 is Sequence ID No:38 and encodes a proteorhodopsin protein according to Sequence ID No:39.
- 68. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m5, and wherein said amplified proteorhodopsin gene from said clone MB100m5 is Sequence ID No:40 and encodes a proteorhodopsin protein according to Sequence ID No:41.
- 69. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m7, and wherein said amplified proteorhodopsin gene from said clone MB100m7 is Sequence ID No:42 and encodes a proteorhodopsin protein according to Sequence ID No:43.
- 70. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m9, and wherein said amplified proteorhodopsin gene from said clone MB100m9 is Sequence ID No:44 and encodes a proteorhodopsin protein according to Sequence ID No:45.
- 71. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m10, and wherein said amplified

proteorhodopsin gene from said clone MB100m10 is Sequence ID No:46 and encodes a proteorhodopsin protein according to Sequence ID No:47.

- 72. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB1, and wherein said amplified proteorhodopsin gene from said clone PALB1 is Sequence ID No:48 and encodes a proteorhodopsin protein according to Sequence ID No:49.
- 73. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB2, and wherein said amplified proteorhodopsin gene from said clone PALB2 is Sequence ID No:50 and encodes a proteorhodopsin protein according to Sequence ID No:51.
- 74. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB5, and wherein said amplified proteorhodopsin gene from said clone PALB5 is Sequence ID No:52 and encodes a proteorhodopsin protein according to Sequence ID No:53.
- 75. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB7, and wherein said amplified proteorhodopsin gene from said clone PALB7 is Sequence ID No:54 and encodes a proteorhodopsin protein according to Sequence ID No:55.
- 76. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB6, and wherein said amplified

proteorhodopsin gene from said clone PALB6 is Sequence ID No:56 and encodes a proteorhodopsin protein according to Sequence ID No:57.

- 77. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB8, and wherein said amplified proteorhodopsin gene from said clone PALB8 is Sequence ID No:58 and encodes a proteorhodopsin protein according to Sequence ID No:59.
- 78. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALE1, and wherein said amplified proteorhodopsin gene from said clone PALE1 is Sequence ID No:60 and encodes a proteorhodopsin protein according to Sequence ID No:61.
- 79. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALE6, and wherein said amplified proteorhodopsin gene from said clone PALE6 is Sequence ID No:62 and encodes a proteorhodopsin protein according to Sequence ID No:63.
- 80. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALE7, and wherein said amplified proteorhodopsin gene from said clone PALE7 is Sequence ID No:64 and encodes a proteorhodopsin protein according to Sequence ID No:65.
- 81. The method of claim 45, wherein said polymerase chain reaction is performed by primers with Sequence ID No:2 and Sequence ID No:3.

- 82. The method of claim 45, further comprising the step of providing a host.
  - 83. The method of claim 82, wherein said host is an artificial membrane system.
  - 84. The method of claim 82, wherein said host is a bacterium.
    - 85. The method of claim 84, wherein said host is a cell membrane preparation of said bacterium.
  - 86. The method of claim 82, wherein said host is an eukaryote.
    - 87. The method of claim 86, wherein said host is a cell membrane preparation of said eukaryote.
- 88. A light-driven energy generator, comprising:
  - (a) a proteorhodopsin protein;
  - (b) a host to correctly fold said proteorhodopsin protein in said host, thereby creating an integrated proteorhodopsin protein; and
  - (c) a source of retinal to bind covalently to said integrated proteorhodopsin protein, thereby creating a light absorbing pigment.
  - 89. The light-driven energy generator of claim 88, wherein said proteorhodopsin protein is encoded by a proteorhodopsin gene retrieved from a genomic fragment of a sample of naturally occurring bacteria.

- 90. The light-driven energy generator of claim 89, wherein said naturally occurring bacteria are marine proteobacteria.
- 91. The light-driven energy generator of claim 89, wherein said naturally occurring bacteria are SAR86 bacteria.
- 92. The light-driven energy generator of claim 89, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.
  - 93. The light-driven energy generator of claim 92, wherein said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.
- 94. The light-driven energy generator of claim 89, wherein said genomic fragment is retrieved from a clone, wherein said clone is a member of the group consisting of BAC31A8, BAC40E8, BAC41B4, BAC64A5, HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7.
- 95. The light-driven energy generator of claim 88, wherein said host is an artificial membrane system.

- 96. The light-driven energy generator of claim 88, wherein said host is a cell membrane obtained from a bacterium.
  - 97. The light-driven energy generator of claim 96, wherein said host is a cell membrane preparation obtained from a bacterium.
- 98. The light-driven energy generator of claim 88, wherein said host is a cell membrane obtained from an eukaryote.
  - 99. The light-driven energy generator of claim 98, wherein said host is a cell membrane preparation obtained from an eukaryote.
- 100. The light-driven energy generator of claim 88, further comprising a light source for illuminating said light absorbing pigment, whereby said energy generator converts light into biochemical energy.
  - 101. The light-driven energy generator of claim 100, wherein said light source is a fast-pulsed light source.
    - 102. The light-driven energy generator of claim 101, wherein said fast-pulsed light source comprises a mechanism for delivering intermittant fast-light pulses at predetermined time intervals.

- 103. The light-driven energy generator of claim 100, wherein said light source is a light source exhibiting different predetermined wavelengths.
- 104. The light-driven energy generator of claim 88, further comprising a mediator for mediating energy generated by said energy generator into chemical, mechanical or electrical energy.
- 105. The light-driven energy generator of claim 88, wherein said proteorhodops in protein is selected to determine an absorption spectra of said light absorbing pigment.
- 106. A method for making a light-driven energy generator, comprising the steps of:
  - (a) providing a proteorhodopsin protein;
  - (b) providing a host to correctly fold said proteorhodopsin protein in said host, thereby creating an integrated proteorhodopsin protein; and
  - (c) providing a source of retinal to bind covalently to said integrated proteorhodopsin protein, thereby creating a light absorbing pigment.
  - 107. The method of claim 106, wherein said proteorhodopsin protein is encoded by a proteorhodopsin gene retrieved from a genomic fragment of a sample of naturally occurring bacteria.
    - 108. The method of claim 107, wherein said naturally occurring bacteria are marine proteobacteria.

- 109. The method of claim 107, wherein said naturally occurring bacteria are SAR86 bacteria.
- 110. The method of claim 107, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.
  - 111. The method of claim 110, wherein said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.
- 112. The method of claim 107, wherein said genomic fragment is retrieved from a clone, wherein said clone is a member of the group consisting of BAC31A8, BAC40E8, BAC41B4, BAC64A5, HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7.
- 113. The method of claim 106, wherein said host is an artificial membrane system.
- 114. The method of claim 106, wherein said host is a cell membrane obtained from a bacterium.

- 115. The method of claim 114, wherein said host is a cell membrane preparation obtained from a bacterium.
- 116. The method of claim 106, wherein said host is a cell membrane obtained from an eukaryote.
  - 117. The method of claim 116, wherein said host is a cell membrane preparation obtained from an eukaryote.
- 118. The method of claim 106, further comprising the step of providing a light source for illuminating said light absorbing pigment, whereby said energy generator converts light into biochemical energy.
  - 119. The method of claim 118, wherein said light source is a fast-pulsed light source.
    - 120. The method of claim 119, wherein said fast-pulsed light source comprises a mechanism for delivering intermittant fast-light pulses at predetermined time intervals.
  - 121. The method of claim 118, wherein said light source is a light source exhibiting different predetermined wavelengths.
- 122. The method of claim 106, further comprising the step of providing a mediator for mediating energy generated by said energy generator into chemical, mechanical or electrical energy.

- 123. The method of claim 106, wherein said proteorhodopsin protein is selected to determine an absorption spectra of said light absorbing pigment.
- 124. A PCR apparatus for amplifying a proteorhodopsin gene from DNA samples of naturally occurring microbial populations using polymerase chain reaction, comprising oligodeoxynucleotide primers with a Watson-Crick base pair complementarity to 5' and 3' ends of said proteorhodopsin gene.
  - 125. The apparatus of claim 124, wherein said primers are according to Sequence ID No:2 and Sequence ID No:3.
- 126. A method of designing PCR primers, comprising the steps of:
  - (a) determining a DNA sequence of a proteorhodopsin gene; and
  - (b) based on said determined DNA sequence in (a), designing oligodeoxynucleotide primers with a Watson-Crick base pair complementarity to said 5' and 3' ends of said proteorhodopsin gene.
  - 127. The method of claim 126, further comprising the step of using said oligodeoxynucleotide primers to amplify said proteorhodopsin gene from DNA samples of naturally occurring microbial populations by polymerase chain reaction.
    - 128. The method of claim 127, further comprising the step of cloning said amplified proteorhodopsin gene into an expression vector.

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129. The method of claim 126, wherein said primers are according to Sequence ID No:2 and Sequence ID No:3.

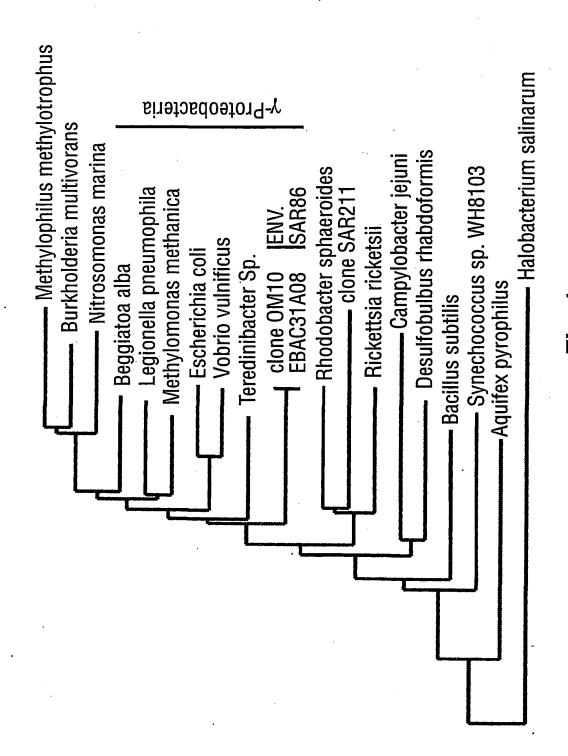


Fig. 1

29

gatattagg aattattact accatgggta

agcattagaa gattctttaa cagc

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cct Pro 15	ggt Gly	. ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tat Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	CCa Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	tta Leu	gca Ala	gct Ala	gat	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggt Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	ggg Gl $_{ m Y}$	gat Asp
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		•				

Figure

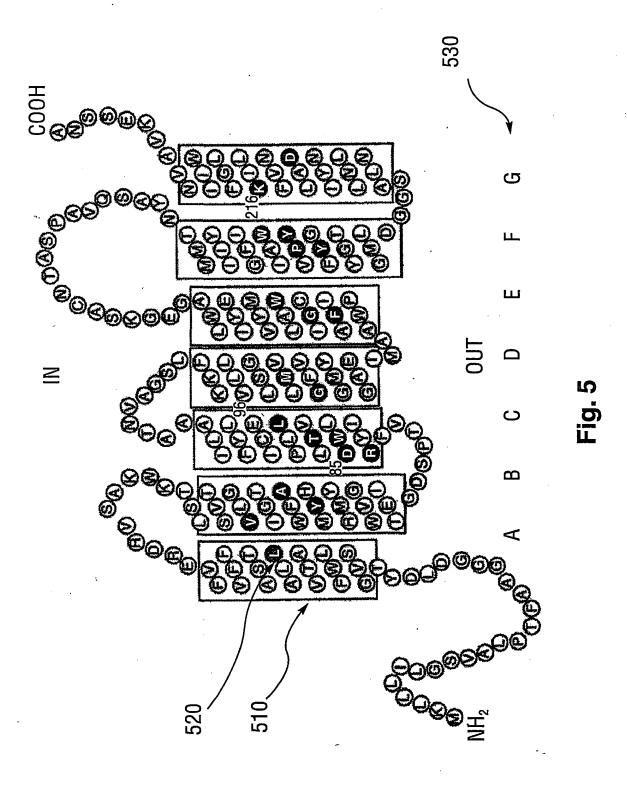
					·	
384	432	480	228	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
aaa t Lys ]	gaa g Glu 2	gct t Ala	gca t Ala ( 175	atg t Met 1	aca g Thr G	atc t Ile 1
aag Lys	ggt Gly	tta	Ser	atg a Met 1	ttc (	ctt ; Leu :
ttt Phe 125	atg Met	tgt Cys	aaa Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	ggg Gl $_{ m Y}$	gga Gly	aac Asn	ggt Gly	tta Leu
tca Ser	ggt Gly	att 11e 155	gaa Glu	tac Tyr	gta Val	aac Asn
gga Gly	ttt Phe	att Ile	gga Gly 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	tta Leu	gtg Val	gcg Ala	gga G1 <u>y</u> 215
act Thr	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gca Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	gct Ala	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt Leu	gtt Val 130	atc Ile	tac Tyr	act Thr	atc Ile	ctg Leu 210
att Ile	cta Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	att Ile	tac Tyr

Figure

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720	7.50
att ata tgg Ile Ile Trp 240	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tat Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val

aat Asn 1



					·	
48	96	144	192	240	. 78	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	ttg Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gag Glu	aca Thr
tta Leu 5	ggt Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35.	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tcg Ser	ggg Gl $_{ m Y}$	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 6

384	432	480	528	576	624	672
ttt aag aaa tta Phe Lys Lys Leu 125	atg ggt gag gca Met Gly Glu Ala	r tgt tta gct tgg r Cys Leu Ala Trp 160	aag gct gca tgt Lys Ala Ala Cys 175	aca atg atg tat Thr Met Met Tyr 190	tat ttc aca ggt Tyr Phe Thr Gly 205	aac ctt atc tat Asn Leu Ile Tyr
gct ggc ctg Ala Gly Leu	ttt ggt tac Phe Gly Tyr 140	gtt att ggg Val Ile Gly 155	gga gaa ggc Gly Glu Gly 170	gct tac aac Ala Tyr Asn	cct gta ggt Pro Val Gly	ctt aac tta Leu Asn Leu 220
aat gtt gct Asn Val Ala 120	atg ctt gtg Met Leu Val 135	ggt gca ttc Gly Ala Phe	cta tgg gct Leu Trp Ala	gtg caa tca Val Gln Ser 185	gca att tat Ala Ile Tyr 200	gga tca gct Gly Ser Ala 215
t gca aca a Ala Thr	ctt gtt Leu Val	gct tgg Ala Trp 150	tat gaa Tyr Glu 165	gt cct gct g er Pro Ala V 80	ggt tgg Gly Trp	gac ggt Asp Gly
att ctt gct gc Ile Leu Ala Al 115	ttg gtt ggt tct Leu Val Gly Ser 130	gga att atg aac Gly Ile Met Asn 145	gta tac atg att Val Tyr Met Ile	aat act gca ag Asn Thr Ala Se 18	ata atc atc ttt Ile Ile Ile Phe 195	tac cta atg ggt Tyr Leu Met Gly 210

Figure

750

720

tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	ct gtt la Val
gct Ala	gct Ala
ctt Leu	gtt Val
gac Asp 225	aat Asn

48	96	144	192	240	2 8 8 7	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	ааа Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	tta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	$\texttt{ggt}\\ \texttt{G1} \texttt{Y}$	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg c	ggt Gly	act Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggt Gly	gct Ala	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	ggg G $1_{ m Y}$	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure

	•					
384	432	480	228	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
aaa t Lys I	gaa g Glu A	gct t Ala I	gca t Ala C 175	atg t Met T	aca g Thr G	atc t Ile T
aag Lys	ggt Gly	tta Leu	Ser	atg Met 1 190	ttc . Phe '	ctt a
ttt Phe 125	atg Met	tgt Cys	aaa Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	ggg G $1 Y$	gga Gly	aac Asn	ggt Gly	tta Leu 220
tca Ser	ggt Gly	att 11e 155	gaa Glu	tac Tyr	gta Val	aac Asn
gga Gly	ttt Phe	att Ile	gga Gly 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt   Val   120	ctt	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct	cta Leu	gtg Val	gcg Ala	gga Gly 215
act Thr	gtt. Val	tgg Trp 150	gaa G1u	gct Ala	tgg Trp	ggt Gly
gct Ala	ctt. Leu	gca Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	gct Ala	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt. . Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
c ctt	a gtt 1 Val 130	a atc	tac . Tyr	act Thr	atc	ctg Leu 210
att Ile	cta Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	att Ile	tac Tyr

Figure '

720	750
tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gat Asp	gtt Val
gct Ala	gct. Ala
ctt Leu	gtt Val
sac Asn 225	aat Asn

48	96	144	192	240	288	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cct Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	ааа Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	aca Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggc Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	gga $_{ m G1Y}$	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

384	432	480	528	576	624	672
aaa ctt Lys Leu	na gca u Ala	it tgg .a Trp 160	a tgt a Cys 5	g gct t Ala	a ggt k Gly	t tat e Tyr
aag Lys	g ggt gaa t Gly Glu	t tta gct s Leu Ala	a tct gca s Ser Ala 175	a atg atg r Met Met 190	t ttc aca r Phe Thr 5	c ctt att n Leu Ile
tta tt Leu Ph 12	tac atg 7 Tyr Met 140	ggg tgt e Gly Cys	ı gga aaa ı Gly Lys	aac aca : Asn Thr	ggt tat Gly Tyr 205	tta aac Leu Asn 220
ggc tca Gly Ser	ttt ggt . Phe Gly	att att Ile Ile 155	gga gaa Gly Glu 170	gct tac Ala Tyr	cct ata Pro Ile	ctt aac Leu Asn
gtt gcc Val Ala 120	ctt gtg Leu Val	gca ttc Ala Phe	tat gct Tyr Ala	caa tca Gln Ser 185	att tat Ile Tyr 200	tca gct Ser Ala
act aat Thr Asn	gtt atg Val Met 135	tgg cct Trp Pro 150	gaa cta Glu Leu	tcg gtt Ser Val	tgg gca Trp Ala	ggt gga Gly Gly 215
gct gca Ala Ala	tct ctt Ser Leu	gca gct Ala Ala	att tat Ile Tyr 165	agt cct Ser Pro 180	ttc ggt Phe Gly	ggt gac Gly Asp
ctt gct Leu Ala 115	gtt ggt Val Gly 130	att atg Ile Met	tac atg Tyr Met	act gca Thr Ala	ata gtc Ile Val 195	cta atg Leu Met 210
att Ile	cta . Leu	gga G1 <u>Y</u> 145	gta Val	aat Asn	atc Ile	tac Tyr

Figure (

750

720

tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta ttt Leu Phe 235	gct Ala
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn 225	aat Asn

50 50 50	100 100 100 100	150 150 150 150	200 200 200 200	250 250 250 250	300 300 300 300
attaggTAGT GTTATTGCAC TTCCTACATT	TTGATGCTAG TGATTACACT GGTGTTTCTT	TTATTAGCAT CTACTGTATT TTTCTTTGTT C	AAAATGGAAA ACATCATTAA CTGTATCTGG	TCTGGCATTA CATGTACATG AGAGGGGTAT	CCAACTGTAT TTAGATACAT TGATTGGTTA
atgggtaaat tattactgat	TGCTGCAGGT GGTGGTGACC	TTTGGTTAGT TACTGCTGCT	GAAAGAGATA GAGTTTCTGC	TCTTGTTACT GGTATTGCTT	GGATTGAAAC TGGTGATTCG
ਜਜਜਜ	51 51 51	101 101 101 101	151 151 151 151	201 201 201 201	251 0 251 251 251
EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64

Figure

350 350 350 350	400 400 400 400	450 450 450 450	500 500 500	550 550 550 550	600 600 600
TTGCTGCTGC	GGTTCTCTTG	GGCTGCATGG .AACT	TTTATGAATTC.	CCTGCTGTGC	TTGGGCGATTA
TACTTAATTC	ATTACTAGTTT.GC.T	CAGGAATCAT	GTATACATGA	TACTGCAAGT	TCATCTTTGG
ATGTGAATTC	TATTTAAGAA .G	ATGGGTGAAGG.	TTTAGCTTGG	CTGCATGTAA	ATGTATATTA A.
CTCTATTAATG	GCTGGATCATCTGGCC	GTTTGGTTAC	TTATTGGGTG	GAAGGAAAAT CGG	CAACACAATG
CTAACAGTTC	AACTAATGTTA T	TTATGCTTGT	CCTGCATTCA GGG	ATGGGCTGGA	AATCAGCTTA
301 301 301 301	351 351 351 351	401 401 401 401	451 451 451 451	501 501 501 501	551 551 551 551
EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64	EBAC31A8 EBAC40 EBAC41 EBAC64

Figure

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650 650 650	700	700	700	750	750	750	750
			•	ttctaatgct		•	•
GTTATTTCAC AGGTTACCTG ATGGGTGACG GTGGATCAGC	TGACTTTGTT AACAAGATTC			ttaaagaatc	•	•	•
AGGTTACCTG	ATAACCTTGC			AATTATATGG AATGTTGCtg	•	•	•
GTTATTTCAC	AACCTTATCT		E.		•	•	•
TATCCTGTAG	TCTTAACTTA			TATTTGGTTT	•	•	
601 601 601	651	651 651	651		701	701	701
EBAC31A8 EBAC40 EBAC41	EBAC31A8	EBAC40 EBAC41	EBAC64	EBAC31A8	EBAC40	EBAC41	EBAC64

EBAC31A8	Н ,	ILGS VIALPTFAAG GGDLDASDYT GVSFWLVTAA	LLASTVEFEV	50
ر ا ا	<del></del>		•	20
 	$\leftarrow$		•	20
EBAC64_1	$\leftarrow$		•	20
1 A S	<u>ր</u>	NEWE TST.TVSGI.VT GIAFWHYMYM BGYMIFTGDS	D#V/FRVTDMT	100
	l 1		T & C + + \ 1 .	) (
$EBAC40_{-}I$	ΣÇ			100
$\mathtt{EBAC41}\_1$	51		• • • • • • •	100
EBAC64_1	51		•	100
EBAC31A8	101	CEF YLILAAATNV AGSLEKKLLV GSLVMLVFGY	MGEAGIMAAW	150
	101	AG	Z	150
EBAC41 1	101		•	4 L
	ι τ Ο (		•	) (
	TOT		•	150
EBAC31A8	151	LAW VYMIYELWAG EGKSACNTAS PAVQSAYNTM	MYIIIFGWAI	200
	151	GV	•	200
	151		•	200
	151	Y S A.	V	200
	201	GYL MGDGGSALNL NLIYNLADFV NKILFGLIIW	NVAVKESSNA	250
EBAC40_1	201		•	250
	201		•	250
	201		•	250

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•						
48	96	144	192	240	288	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lýs 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	ttg Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	acc Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gag Glu	aca Thr
tta Leu 5	ggt Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa G1u	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tcg Ser	ggg G $1_{ m Y}$	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 11

WO 01/83701		PCT/US01/14394
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384	432	480	528	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
aaa Lys	gag Glu	gct Ala	gca Ala 175	atg Met	aca Thr	atc Ile
aag Lys	ggt Gly	tta Leu	gct Ala	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	aag Lys	aca Thr	tat Tyr 205	aac Asn
ctg Leu	tac Tyr 140	ggg Gl $_{ m Y}$	ggc Gly	aac Asn	ggt Gly	tta Leu 220
ggc $_{\rm G1Y}$	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gct Ala	ttt Phe	gtt Val	gga Gly 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	ggt Gly	cta Leu	gtg Val	gca Ala	gga G1y 215
аса Тћг	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	aac Asn	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt Leu	gtt Val 130	att Ile	tac Tyr	act Thr	atc Ile	cta Leu 210
att Ile	ttg Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	ata Ile	tac Tyr

Figure 11

720	750
tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt	gtt Val
ac sn : 25	at sn

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WO 01/8	33701		24/108	` }		PCT/US01/1
48	96	144	192	240	288	336
t aaa tta tta ctg ata tta ggt agt gct att gca ctt cca tca Y Lys Leu Leu Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser 10	t gct gct ggt ggc gat cta gat ata agt gat act gtt ggt gtt a Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20	c tgg ctg gtt aca gct ggt atg tta gcg gca act gtg ttc ttt e Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe 35	a gaa aga gac caa gtc agc gct aag tgg aaa act tca ctt gct 1 Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Ala 55	ggt tta att act ggt ata gct ttt tgg cat tat ctc tat atg Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 70	gtt tgg ata gac act ggt gat acc cca aca gta ttc aga tat Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 90	tgg tta tta act gtt cca tta caa atg gtt gag ttc tat cta Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100
atg ggt Met Gly 1	ttt gct Phe Ala	tca ttc Ser Phe	ttt gta Phe Val 50	gta tct Val Ser 65	aga ggt Arg Gly	att gat Ile Asp

Figure 12

PCT/US01/14394

WO 01/83701	
,	25/108

384	432	480	528	576	624	672
agt gtt gct gct tca tta ttt aag aag ctt Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 120	atg tta ggt gct gga ttt gca ggc gaa gct Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 135	cct gct ttc att att ggt atg gct gga tgg Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 155	cta tat atg ggt gaa ggt aag gct gct gta Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 170	gtt aac tct gca tac aac gca atg atg Val Asn Ser Ala Tyr Asn Ala Met Met Met 185	gca att tat cct gct gga tat gct gct ggt Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 200	ggt gta tac gct tca aac tta aac ctt ata Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 215
tgt aca Cys Thr	tta gta Leu Val	gta tta Val Leu 150	tat gag Tyr Glu 165	cct gct Pro Ala	gga tgg Gly Trp	ggc gaa Gly Glu
gct Ala	tca Ser	cct Pro	att t Ile 7	agt c Ser I 180	gtt g Val G	ggt g Gly G
gct 1 Ala 115	ggt Gly	gct Ala	atg Met	gca	gtt Val 195	atg Met
ctt	gct   Ala   130	tta Teu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	gga Gly 145	tta Leu	agt Ser	att Ile	tac Tyr

Figure 12

PCT/US01/14394

753

720

	,
att Ile 240	
atc Ile	
ttg Leu	
ggt Gly	
ttt Phe	
cta Leu 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ctt Leu 230	aaa Lys
gac Asp	gtt Val 245
gcc Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

48	96	144	192	240	288	336
cca tca Pro Ser 15	ggt gtt Gly Val	ttc ttt Phe Phe	ctt act Leu Thr	tac atg Tyr Met 80	aga tat Arg Tyr 95	tat cta Tyr Leu
ctt Leu	gtt Val 30	gta Val	tca Ser	ctc Leu	ttt Phe	ttc Phe 110
att gca Ile Ala	gat act Asp Thr	gca act Ala Thr 45	aaa act Lys Thr 60	cat tat His Tyr	aca gta Thr Val	gtt gag Val Glu
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
ggt agt Gly Ser 10	gat ata Asp Ile 25	atg tta Met Leu	gct aag Ala Lys	gct ttt Ala Phe	gat aca Asp Thr 90	tta caa Leu Gln 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ctg ata Leu Ile	ggc gat Gly Asp	aca gct Thr Ala	caa gtc Gln Val 55	act ggt Thr Gly 70	gat act Asp Thr	act gtt Thr Val
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
aaa tta Lys Leu	gct gct Ala Ala 20	tgg ctg Trp Leu 35	gaa aga Glu Arg	ggt tta Gly Leu	gtt tgg Val Trp	tgg tta Trp Leu 100
ggt Gly	gct Ala	ttc Phe	gta Val 50	tct Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 13

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384	432	480	528	576	624	672
ctt Leu	gct Ala	tgg Trp 160	gta .val	aag Lys	ggt Gly	ata Ile
aag Lys	gaa Glu	gga Gly	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta Leu
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aac Asn	gga Gly	aac Asn 220
tca Ser	gga Gly	att Ile 155	gaa Glu	tac Tyr	gct. Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	cat His	aac Asn	att Ile 200	gta Val
agt Ser	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gac Asp
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga $\mathtt{G1}_{Y}$	ggt Gly
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	att Ile	agt Ser
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att 11e	cta Leu 210
att Ile	cta Leu	ggt Gly 145	tta Leu	agt Ser	att Ile	tac Tyr
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720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	
cta t Leu P 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

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48	96	144	192	240	288	336
						,
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	ρK	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	gtg Val
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	gac Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tct Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 14

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384	432	480	528	576	624	672
g ctt s Leu	a gct u Ala	a tgg Y Trp 160	t gta a Val	atg Met	ggt a Gly	ata 1 Ile
g aag s Lys	gaa Glu	gga Gly	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta Leu
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aac Asn	gga Gly	aac Asn 220
tca Ser	gga Gly	att Ile 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg. Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	tat Tyr	aac Asn	att Ile 200	gta Val
agt Ser	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gaa Glu
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga ${ t G1}Y$	ggc Gly
gct Ala	tca Ser	cct Pro	att. Hle	agt Ser 180	gtt Val	ggt Gly
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	gga G1Y 145	tta Leu	agt Ser	att Ile	tac Tyr

Figure 14

720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	
cta Leu 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

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48	96	1.44	192	240	288	336
tca Ser	gtt Val	ttt	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	ga <i>c</i> Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tot Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 1

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384	432	480	528	576	624	672
ctt gct gct tgt aca aat gtt gct gct tca tta ttt aag aag ctt Leu Ala Ala Cys Thr Asn Val Ala Ala Ser Leu Phe Lys Lys Leu 115	gct ggt tca tta gta atg tta ggt gct gga ttt gca ggc gaa gct Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130	ttg gct cct gta tgg cct gct ttc att att ggt atg gct gga tgg Leu Ala Pro Val Trp Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 150	tac atg att tat gag cta tat atg ggt gaa ggt aag gct gct gta Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 175	act gca agt cct gct gtt aac tct gca tac aac gca atg atg gtg Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Wet Val 180	t gtt gtt gga tgg gca att tat cct gct gga tat gct gct ggt e Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 205	cta atg ggt ggc gaa ggt gta tac gct tca aac tta aac ctt ata Leu Met Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210
att o Ile I	cta gc Leu Al 13	gga t Gly L 145	tta t Leu T	agt a Ser T	att at Ile Il	tac c Tyr L

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720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	
ctt gtt aac aag att cta t Leu Val Asn Lys Ile Leu 1 230	aaa gaa tct tct aat gct Lys Glu Ser Ser Asn Ala 250
tat aac ctt gcc gac Tyr Asn Leu Ala Asp 225	tgg aat gtt gct gtt Trp Asn Val Ala Val

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48	96	144	192	240	2 88	336
aca Thr	gtt Val	ttc	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	ttg Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gag Glu	aca Thr
tta Leu 5	ggt Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	ggg Gly	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

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384	432	480	528	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	gct	ggt Gly	tat Tyr
aaa Lys	gag Glu	gct Ala	gca Ala 175	atg Met	aca Thr	att Ile
aag Lys	ggt Gly	tta Leu	ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	ааа Lys	aca Thr	tat Tyr 205	aac Asn
ctg Leu	tac Tyr 140	ggg G $1 Y$	gga ${ t G1}Y$	aac Asn	ggt Gly	tta Leu 220
ggc Gly	ggt Gly	att I1e 155	gaa Glu	tac Tyr	gta Val	aac Asn
gct Ala	ttt Phe	att Ile	gga Gly 170	gct Ala	cct	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tat Tyr	caa G1n	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	gga G1 <u>y</u> 215
aca Thr	gtt Val	tgg Trp 150	gaa Glu	tcg Ser	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct	ggt Gly	gac Asp
gct Ala	tct Ser	aac Asn	att Ile	agt Ser 180	ttc Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	gtc Val 195	atg Met
ctt Leu	gtt Val 130	att Ile	tac Tyr	act Thr	ata Ile	cta Leu 210
att Ile	ttg Leu	gga G1Y 145	gta Val	aat Asn	atc Ile	tac TYr

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720	750
t ata tgg e Ile Trp 240	
tta att Leu Ile	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
8 BC	at

48	96 ,	144	192	240	288	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	au	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	tta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt $_{ m G1Y}$	act Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggt Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	ggg G $1_Y$	gat
atg Met 1	ttt	tct Ser	ttt	gta Val 65	aga Arg	att Ile

Figure 17

384	432	480	528	576	624	672.
tta Leu	gca Ala	tgg Trp 160	tgt Cys	atg Met	ggt Gly	tat Tyr
ааа Lys	gaa Glu	gct Ala	gcg Ala 175	atg Met	aca Thr	atc Ile
aag Lys	ggt Gly	tta Leu	gct Ala	atg Met 190	ttc Phe	ctt Leu
ttť Phe 125	atg Met	tgt Cys	ааа Lys	aca Thr	tat Tyr 205	aac Asn
ctg Leu	tac Tyr 140	ggg Gl $_{ m Y}$	gga Gly	aac Asn	ggt Gly	tta Leu 220
ggc Gly	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gct Ala	ttt Phe	gtt Val	gga G1y 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	ctt Leu	tca Ser 185	tat Tyr	gca Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	cag Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	ggt Gly	ctt Leu	gtt Val	gca Ala	gga G1y 215
act Thr	gtt Val	tgg Trp 150	gag Glu	gct Ala	tgg Trp	ggt Gly
gct Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	aac Asn	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met ~
ctt Leu	gtt Val 130	att Ile	tac Tyr	aca Thr	atc Ile	cta Leu 210
att Ile	ttg Leu	gga Gly 145	gta Val	aat Asn	atc Ile	tac Tyr

Figure 17

750

720

tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn 225	aat Asn

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48	96	144	2	0,	ω,	9
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aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tog Ser 90	tta Leu
ggt Gly	gat Asp 25	tta Leu	gca Ala	gct Alà	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggt $_{ m G1Y}$	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	ggg Gly	gat Asp
atg Met 1	ttt Phe	tat Ser	ttt Phe	gta Val 65	aga Arg	att Ile
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Figure 18

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384	432	480	528	576	624	672
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et 51		<b>—</b> 0 —				
tta Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
ааа Lys	gag Glu	gct Ala	gca Ala 175	atg Met	aca Thr	atc Ile
aag Lys	ggt Gly	tta Leu	gct Ala	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	aag Lys	aca Thr	tat Tyr 205	aac Asn
ctg Leu	tac Tyr 140	ggg Gly	ggc Gly	aac Asn	ggt Gly	tta Leu 220
ggc Gly	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gct Ala	ttt Phe	gtt Val	gga Gly 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	ggt Gly	cta Leu	gtg Val	gca Ala	gga G1y 215
act Thr	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	aac Asn	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt	gtt Val 130	att Ile	tac Tyr	act Thr	atc Ile	cta Leu 210
att Ile	ttg Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	ata Ile	tac Tyr

720	750
ata tgg Ile Trp 240	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt	gtt Val

aac Asn 225

aat Asn

48	96	144	192	240	288	336
ctt cct aca Leu Pro Thr 15	act ggt gtt Thr Gly Val 30	gta ttt ttc. Val Phe Phe	tca tta act Ser Leu Thr	atg tac atg Met Tyr Met 80	ttt aga tac Phe Arg Tyr 95	ttc tac tta Phe Tyr Leu 110
att gca ct Ile Ala Le	gat tac ac Asp Tyr Th 30	tct act gt Ser Thr Va 45	aaa aca tc Lys Thr S6 60	cat tac at His Tyr Me	act gta tt Thr Val Pł	tgt gaa tt Cys Glu Pl 11
agt gtt at Ser Val II 10	gct agt ge Ala Ser As	tta gca tc Leu Ala Se	aaa tgg aa Lys Trp Ly 6(	ttc tgg ca Phe Trp Hi 75	tcg cca ac Ser Pro Th 90	tta ata tç Leu Ile C <u>y</u>
tta ggt ag Leu Gly Se 10	ctt gat go Leu Asp A 25	gct cta ti Ala Leu Le 40	tct gca a? Ser Ala Ly	att gct ti Ile Ala Pl	ggt gat to Gly Asp S6 9(	cct cta ti Pro Leu Le 105
ctg ata t Leu Ile L	ggt gac c Gly Asp L	aca gct g Thr Ala A	aga gtt to Arg Val So 55	act ggt ai Thr Gly I: 70	gaa act ge Glu Thr G	aca gtt co Thr Val P
tta tta c Leu Leu L 5	ggt ggc g Gly Gly G 20	tta gtt a Leu Val T	aga gat a Arg Asp A	ctt gtt a Leu Val T	tgg att g Trp Ile G 85	tta cta a Leu Leu T 100
ggt aaa t Gly Lys L	gct gca g Ala Ala G 2	ttt tgg t Phe Trp L 35	gtt gaa a Val Glu A 50	tct ggt c Ser Gly L	gg gta ly Val	gat tgg t Asp Trp L
atg g Met G 1	ttt g Phe P	tct t Ser E	ttt g Phe V	gta t Val S 65	aga g Arg G	att g Ile A

Figure 1

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384	432	480	228	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	gct Ala	ggt Gly	tat Tyr
aaa Lys	gaa Glu	gct Ala	gca Ala 175	atg Met	aca Thr	att Ile
aag Lys	ggt Gly	tta Leu	tct Ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	ааа Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	ggg G $1 Y$	gga Gly	aac Asn	ggt Gly	tta Leu 220
tca Ser	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gga Gly	ttt Phe	att Ile	gga G1Y 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tat Tyr	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggg Gly 215
act	gtt Val	tgg Trp 150	gaa Glu	tcg Ser	tgg Trp	ggt Gly
gct Ala	ctt Leu	gca Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	gct Ala	att Ile	agt Ser 180	ttc Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	gtc Val 195	atg Met
ctt Leu	gtt Val 130	att Ile	tac Tyr	act Thr	ata Ile	cta Leu 210
att Ile	cta Leu	caa Gln 145	gta Val	aat Asn	atc Ile	tac Tyr

Figure 19

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720	750
ctt ggt tta att ata tgg Leu Gly Leu Ile Ile Trp 235	
aag att cta Lys Ile Leu	tct aat gct Ser Asn Ala 250
c ttt gtt aac p Phe Val Asn 230	t aaa gaa tct 1 Lys Glu Ser 245
aac ctt gct ga Asn Leu Ala Asj 225	aat gtt gct gtt Asn Val Ala Val

48	96	144	192	240	288	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	Ser	aaa Lys 60	cat	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	ааа Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	tta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggt Gl <u>y</u>	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	ggg Gl $_{ m Y}$	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att 11e

Figure 20

384	432	480	528	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
aaa Lys	gaa Glu	gct Ala	gca Ala 175	atg Met	aca Thr	atc Ile
aag Lys	ggt Gly	tta Leu	Ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	ааа Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	ggg G $1_{ m Y}$	gga $_{ m G1Y}$	aac Asn	ggt Gly	tta Leu 220
tca Ser	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gga Gly	ttt Phe	att Ile	gga Gly 170	gcc Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	tta Leu	gtg Val	gcg Ala	gga G1 <u>y</u> 215
gct Ala	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gca Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tat Ser	gct Ala	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt Leu	gtt Val 130	atc Ile	tac Tyr	act Thr	atc Ile	ttg Leu 210
att Ile	cta Leu	gga Gly 145	gta Val	aat Asn	att Ile	tac Tyr

Figure 20

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720	750
ata tgg Ile Trp 240	
att Ile J	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala
att cta Ile Leu	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt aac Val·Asn 230	gaa G1u
rtt Phe	aaa Lys
gac Asp	gtt Val
gct Ala	gct Ala
Leu	gtt Val
ac sn 25	at sn

WO 01/83701		PCT/US01/14394
	51/108	

		•				
48	96	144	192	240	288	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	ааа Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	tta Leu 105
ata Ile	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	aca Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta ctg Leu Leu 5	ggc Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	Ser	gga G1y	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 21

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384	432	480	528	576	624	672
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ctt Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
aaa Lys	gaa Glu	gct Ala	gca Ala 175	atg Met	аса Тћг	atc Ile
aag Lys	ggt Gly	tta Leu	tct Ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	aaa Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	ggg Gly	gga Gly	aac Asn	ggt Gly	tta Leu 220
tca Ser	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
ggc Gly	ttt Phe	att Ile	gga Gly 170	gct Ala	cct Pro	ctt Leu
gcc Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tat Tyr	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	cta Leu	gtg Val	gcg Ala	gga Gly 215
act Thr	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt	gac Asp
gct Ala	tct Ser	gca Ala	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	gtc Val 195	atg Met
ctt Leu	gtt Val 130	att Ile	tat Tyr	aca Thr	atc Ile	ctg Leu 210
att Ile	cta Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	att Ile	tac Tyr

Figure 21

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720	750
ggt tta att ata tgg Gly Leu Ile Ile Trp 240	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa G1u
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
ac sn 25	at

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WO 01/83	3701		54/108			PCT/US01/14
48	96	144	192	240	288	336
ta tta ggt agt gtt att gca ctt cct aca le Leu Gly Ser Val Ile Ala Leu Pro Thr 10	gac ctt gat gct agt gat tac act ggt gtt Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 25	gct gct cta tta gca tct act gta ttt ttc Ala Ala Leu Leu Ala Ser Thr Val Phe 40	gtt tct gca aaa tgg aaa aca tca tta act Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 55	gt att gct ttc tgg cat tac atg tac atg ly Ile Ala Phe Trp His Tyr Met Tyr Met 75	act ggt gat tcg cca act gta ttt aga tac Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 90	gtt cct cta ttg ata tgt gaa ttc tac tta Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 105
atg ggt aaa tta tta ctg at Met Gly Lys Leu Leu Ll 1	ttt gct gca ggt ggt ggt ga Phe Ala Ala Gly Gly As 20	tct ttt tgg tta gtt act gc Ser Phe Trp Leu Val Thr Al 35	ttt gtt gaa aga gat aga gt Phe Val Glu Arg Asp Arg Va 50	gta tcg ggt ctt gtt act gg Val Ser Gly Leu Val Thr Gl 65	aga ggg gta tgg att gag ac Arg Gly Val Trp Ile Glu Th 85	att gat tgg tta cta aca gt Ile Asp Trp Leu Leu Thr Va

Figure 22

	7701		55/108			101,0001,11
384	432	480	528	576	624	672
tta Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Ty <i>r</i>
aaa Lys	gag Glu	gct Ala	gca Ala 175	atg Met	aca Thr	atc Ile
aag Lys	ggt Gly	tta Leu	gct Ala	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	aag Lys	aca Thr	tat Tyr 205	aac Asn
ctg Leu	tac Tyr 140	ggg Gly	ggc Gly	aac Asn	ggt Gly	tta Leu 220
ggc Gly	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gct Ala	ttt Phe	gtt Val	gga G1 <u>y</u> 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	ggt Gly	cta Leu	gtg Val	gca Ala	gga G1y 215
aca Thr	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	aac Asn	att Ile	agt Ser 180	ttt Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt Leu	gtt Val 130	att Ile	tac Tyr	act Thr	atc Ile	cta Leu 210
att Ile	ttg Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	ata Ile	tac Tyr

750

720

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tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
aat Asn	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
rtt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
CCT	gtt Val
aac Asn 225	aat Asn

WO 01/8	3701		57/108			PC1/USU1/14
48	96	144	. 192	240	288	336
g ggt aaa tta tta cgg ata tta ggt agt gtt att gca ctt cct aca t Gly Lys Leu Leu Arg Ile Leu Gly Ser Val Ile Ala Leu Pro Thr 5	ttt gct gca ggt ggc ggt gac ctt gat gct agt gat tac act ggt gtt Phe Ala Ala Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 25	t ttt tgg tta gtt aca gct gct cta tta gca tct act gta ttt ttc r Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe 35	t gtt gaa aga gat aga gtt tct gca aaa tgg aaa aca tca tta act e Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50	a tct ggt ctt gtt act ggt att gct ttc tgg cat tac atg tat atg l Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 70	a gga gta tgg att gaa act ggt gat tcg cca act gta ttt aga tac g Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 90	tt gat tgg tta cta aca gtt cct tta tta ata tgt gaa ttc tac tta le Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100
atg Met 1	th Th	tct Ser	ttt Phe	gta Val 65	aga Arg	øН

Figure 23

WO 01/83701		PCT/US01/14394
	58/108	

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384	432	480	528	576	624	672
tca tta ttt aag aaa tta Ser Leu Phe Lys Lys Leu 125	ggt tac atg ggt gaa gca . Gly Tyr Met Gly Glu Ala 140	att ggg tgt tta gct tgg Ile Gly Cys Leu Ala Trp 155	gaa gga aaa tct gca tgt Glu Gly Lys Ser Ala Cys 175	tac aac aca atg atg tat Tyr Asn Thr Met Met Tyr 190	gta ggt tat ttc aca ggt Val Gly Tyr Phe Thr Gly 205	aac tta aac ctt atc tat Asn Leu Asn Leu Ile Tyr 220
ot gct gca act aat gtt gct gga la Ala Ala Thr Asn Val Ala Gly 15	gt tct ctt gtt atg ctt gtg ttt ly Ser Leu Val Met Leu Val Phe 135	og gct gca tgg cct gca ttc atter st Ala Ala Trp Pro Ala Phe Ile 150	og att tat gaa cta tgg gct gga et Ile Tyr Glu Leu Trp Ala Gly 165	ta agt cct gct gtg caa tca gct la Ser Pro Ala Val Gln Ser Ala 180	c gtt ggt tgg gcg att tat cct le Val Gly Trp Ala Ile Tyr Pro 35	ig ggt gac ggt gga tca gct ctt et Gly Asp Gly Gly Ser Ala Leu 215
att ctt gct Ile Leu Ala 115	cta gtt gg Leu Val Gl 130	gga atc atg Gly Ile Met 145	gta tac atg Val Tyr Met	aat act gca Asn Thr Ala	atc atc at Ile Ile Il	tac ctg atg Tyr Leu Met 210

Figure 23

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720	750
ttt ggt tta att ata tgg Phe Gly Leu Ile Ile Trp 235	
ac aag att cta sn Lys Ile Leu	tct tct aat gct Ser Ser Asn Ala 250
ac ctt gct gac ttt gtt a sn Leu Ala Asp Phe Val A 25	at gtt gct gtt aaa gaa sn Val Ala Val Lys Glu 245
8 K C	დ ≮

48	96	144	192	240	88	336
	•		Н	0	7	m
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt	tta Leu	tac Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	aca Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggc Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	gga ${ t G1}{ t Y}$	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 24

384	432	480	528	576	624	672
ctt Leu	gca Ala	tgg Trp 160	tgt Cys	gct Ala	ggt Gly	tat Tyr
aaa Lys	gaa Glu	gct Ala	gca Ala 175	atg Met	aca Thr	att Ile
aag Lys	ggt Gly	tta Leu	tct Ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	aaa Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	999 G1y	gga Gly	aac Asn	ggt Gly	tta Leu 220
tca Ser	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
ggc Gly	ttt Phe	att Ile	gga Gly 170	gct Ala	cct Pro	ctt Leu
gcc Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tat Tyr	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	gga G1 <u>y</u> 215
act Thr	gtt Val	tgg Trp 150	gaa Glu	tcg Ser	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	gca Ala	att Ile	agt Ser 180	ttc Phe	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	gtc Val 195	atg Met
ctt Leu	gtt Val 130	att Ile	tac Tyr	act Thr	ata Ile	cta Leu 210
att Ile	cta Leu	gga G1y 145	gta Val	aat Asn	atc Ile	tac Tyr

Figure 24

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720	750
tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
25 25	at Sn

48	96	144	192	240	288	336
aca Thr	gtt Val	ttc Phe	act Thr	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tac Tyr	aga Arg 95	tac Tyr
cťt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att Ile	gat Asp	tat	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gca Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	tta Leu	gca Ala	gct Ala	gat Asp	cta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	act Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggt Gly	gtt Val	gat Asp	gtt Val	att Ile 85	cta Leu
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	$\tt ggg \\ \tt G1y$	gat Asp
atg Met 1	ttt Phe	tat Ser	ttt Phe	gta Val 65	aga Arg	att Ile
,						

Figure 25

		04/100			
432	480	528	576	624	672
ta gtt ggt tct ctt gtt atg ctt gtg ttt ggt tac atg ggt gaa gca eu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130	a att atg gca gct t y Ile Met Ala Ala T 5	ta tac atg att tat gaa cta tat gct gga gaa gga aaa tct gca tgt 11 Tyr Met Ile Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys 170	it act gca agt cct tcg gtt caa tca gct tac aac aca atg atg gct in Thr Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala 180	c ata gtc ttc ggt tgg gca att tat cct gta ggt tat ttc aca ggt e Ile Val Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly 205	tac cta atg ggt gac ggt gga tca gct ctt aac tta aac ctt att tat Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210
OH	01 0 11	حز ان	וט אנן	₩ H	ъH
	ggt tct ctt gtt atg ctt gtg ttt ggt tac atg ggt gaa gca 43 Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 135	gtt ggt tct ctt gtt atg ctt gtg ttt ggt tac atg ggt gaa gca 43 Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130  att atg gca gct tgg cct gca ttc att att ggg tgt tta gct tgg 160 Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 150 150	yal ggt tct ctt gtt atg ctt gtg ttt ggt tac atg ggt gaa gca Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130  att atg gca gct tgg cct gca ttc att att ggg tgt tta gct tgg 48  Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 160  tac atg att tat gaa cta tat gct gga gaa gga aaa tct gca tgt 77  tac atg att tat gaa cta tat gct gga gaa gga aaa tct gca tgt 77  Tyr Met Ile Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys 175	a gft ggt tct ctt gft atg ctt gtg ttt ggt tac atg ggt gaa gca 132  130  a att atg gca gct tgg cct gca ttc att att ggg tgt tta gct tgg 480  y lle Met Ala Ala Trp Pro Ala Phe lle lle Gly Cys Leu Ala Trp 160  a tac atg att tat gaa cta tat gct gga gaa gga aaa tct gca tgt 528  1 Tyr Met lle Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys 165  t act gca agt cct tcg gtt caa tca gct tac aac aca atg atg gct 175  t act gca agt cct tcg gtt caa tca gct tac aac aca atg atg gct 180  t Thr Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala 180  180	The Met Ice of the ten val Phe Gly Tyr Met Gly Glu Ala att atg gra gra gra gra gra Iss att atg gra gra gra try Pro Ala Phe Ile Ile Gly Cys Leu Ala Try Iss att at gra gra gra gra gra gra gra try Ala Gly Glu Gly Lys Ser Ala Cys Iss Iss at gra gra trat gra gra gra gra ara try Glu Ice Tyr Ala Gly Glu Gly Lys Ser Ala Cys Iss Iss at gra gra ara try Glu Ice Tyr Ala Gly Glu Gly Iys Ser Ala Cys Iss Iss at gra gra ara try Asn Thr Met Met Ala Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Gly Tyr Asn Thr Met Its ara gra att trat crt gra gra trat tro ara gra Iss Iss Iss Iss Iss Iss Iss Iss Iss Is

Figure 25

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720	750
tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gct Ala
aac Asn 225	aat Asn

			00/100			
48	96	144	192	240	2 8 8	336
a ctt cct aca a Leu Pro Thr 15	act ggt gtt Thr Gly Val	gta ttt ttc Val Phe Phe	tca tta act Ser Leu Thr	atg tat atg Met Tyr Met 80	ttt aga tac Phe Arg Tyr 95	ttc tac tta   Phe Tyr Leu   110
t gtt att gca r Val Ile Ala	t agt gat tac a Ser Asp Tyr	a gca tct act u Ala Ser Thr 45	a tgg aaa aca s Trp Lys Thr 60	c tgg cat tac e Trp His Tyr 75	g cca act gta r Pro Thr Val	a ata tgt gaa ı Ile Cys Glu
tta ggt agt Leu Gly Ser 10	ctt gat gct Leu Asp Ala 25	gct tta tta Ala Leu Leu 40	tct gca aaa Ser Ala Lys	att gct ttc Ile Ala Phe	ggt gat tcg Gly Asp Ser 90	cct tta tta Pro Leu Leu 105
tta ctg ata Leu Leu Ile 5	ggt ggt gac Gly Gly Asp	gtt act gct Val Thr Ala	gat aga gtt Asp Arg Val 55	gtt act ggt Val Thr Gly 70	att gaa act Ile Glu Thr 85	cta aca gtt Leu Thr Val
atg ggt aaa tta Met Gly Lys Leu 1	ttt gct gca ggt Phe Ala Ala Gly 20	tct ttt tgg tta Ser Phe Trp Leu 35	ttt gtt gaa aga Phe Val Glu Arg 50	gta tct ggt ctt Val Ser Gly Leu 65	aga ggg gta tgg Arg Gly Val Trp	ata gat tgg tta Ile Asp Trp Leu 100
				•	- •	

Figure 26

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PCT	/11801	/14394

WO 01/8	3701		67/108			PCT/US01/14
384	432	480	528	576	624	672
tta Leu	. gca . Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Ty <i>r</i>
ааа Lys	gaa Glu	gct Ala	gca Ala 175	atg Met	aca Thr	att Ile
aag Lys	ggt Gly	tta Leu	tct Ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	aaa Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	ggg Gļy	gga Gly	aac Asn	ggt Gly	tta Leu 220
tca Ser	ggt Gly	att Ile 155	gaa Glu	tac Tyr	gta Val	aac Asn
gga Gly	ttt Phe	att Ile	gga G1y 170	gct Ala	cct Pro	ctt Leu
gct Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gca Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	caa Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	cta Leu	gtg Val	gcg Ala	gga G1y 215
act Thr	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gca Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	gct Ala	att Ile	agt Ser 180	ttt Phe	ggt Gly
gcc Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt Leu	gtt Val 130	atc Ile	tac Tyr	act Thr	atc Ile	ctt Leu 210
att Ile	ctt Leu	gga Gly 145	gta Val	aat Asn	atc Ile	tac Tyr
•						

Figure 26

7.7	75
tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
cta ttt Leu Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aac aag Asn Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa G1u
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
ac sn 25	at sn

figure 20

•						
48	96	144	192	240	. 8 8	336
aca Thr	gtt Val	ttc Phe	act	atg Met 80	tac Tyr	tta Leu
cct Pro 15	ggt Gly	ttt Phe	tta Leu	tat Tyr	aga Arg 95	tac Tyr
ctt Leu	act Thr 30	gta Val	tca Ser	atg Met	ttt Phe	ttc Phe 110
gca Ala	tac Tyr	act Thr 45	aca Thr	tac Tyr	gta Val	gaa Glu
att 11e	gat Asp	tct Ser	aaa Lys 60	cat His	act Thr	tgt Cys
gtt Val	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	ata Ile
agt Ser 10	gct Ala	tta Leu	aaa Lys	ttc Phe	tcg Ser 90	tta Leu
ggt Gly	gat Asp 25	cta Leu	gca Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	ctt Leu	gct Ala 40	tct Ser	att Ile	ggt Gly	cct Pro
ata Ile	gac Asp	gct Ala	gtt Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggt Gly	aca Thr	aga Arg	act Thr 70	gaa Glu	aca Thr
tta Leu 5	ggc $_{ m G1Y}$	gtt Val	gat Asp	gtt Val	att Ile 85	cta
tta Leu	ggt Gly 20	tta Leu	aga Arg	ctt Leu	tgg Trp	tta Leu 100
aaa Lys	gca Ala	tgg Trp 35	gaa Glu	ggt Gly	gta Val	tgg Trp
ggt Gly	gct Ala	ttt Phe	gtt Val 50	tct Ser	gga Gly	gat Asp
atg Met 1	ttt Phe	tct Ser	ttt Phe	gta Val 65	aga Arg	att Ile

WO 01/83701		PCT/US01/14394
	70/108	

4	7	0	ω .	9	4	0
38	43	48	52	57	62,	67.
ctt Leu	gca Ala	tgg Trp 160	tgt Cys	tat Tyr	ggt Gly	tat Tyr
aaa Lys	gaa Glu	gca Ala	gca Ala 175	atg Met	aca Thr	att Ile
aag Lys	ggt Gly	tta Leu	tct Ser	atg Met 190	ttc Phe	ctt Leu
ttt Phe 125	atg Met	tgt Cys	ааа Lys	aca Thr	tat Tyr 205	aac Asn
tta Leu	tac Tyr 140	gga $_{ m GLY}$	gga Gly	aac Asn	ggt Gly	cta Leu 220
tca Ser	ggt Gly	gtt Val 155	gaa Glu	tac Tyr		
ggc Gly	ttt Phe	atc Ile	ggt Gly 170	gct Ala	cct gta Pro Val	cttjaat Leu Asn
gcc Ala	gtg Val	ttc Phe	gct Ala	tca Ser 185	tat Tyr	gct Ala
gtt Val 120	ctt Leu	gca Ala	tgg Trp	cag Gln	att Ile 200	tca Ser
aat Asn	atg Met 135	cct Pro	cta Leu	gta Val	gca Ala	gga Gly 215
act Thr	gtt Val	tgg Trp 150	gaa Glu	gct Ala	tgg Trp	ggt Gly
gca Ala	ctt Leu	gct Ala	tat Tyr 165	cct Pro	ggt Gly	gac Asp
gct Ala	tct Ser	gcg Ala	att Ile	agt Ser 180	gtt Val	ggt Gly
gct Ala 115	ggt Gly	atg Met	atg Met	gca Ala	atc Ile 195	atg Met
ctt Leu	gtt Val 130	ata Ile	tat Tyr	act Thr	atc Ile	cta Leu 210
att Ile	cta Leu	gga G1 <u>y</u> 145	gta Val	aat Asn	atc Ile	tac Tyr

Figure 27

750

720

tgg Trp 240	
ata Ile	
att Ile	
tta Leu	
ggt Gly	
ttt Phe 235	
cta Leu	gct Ala 250
att Ile	aat Asn
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val 230	gaa Glu
ttt Phe	aaa Lys 245
gac Asp	gtt Val
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn 225	aat Asn

WO 01/83701		PCT/US01/14394
	72/108	

48	96	144	192	240	288	336
gca ctt cca tca Ala Leu Pro Ser 15	act gtt ggt gtt Thr Val Gly Val 30	act gta ttc ttt Thr Val Phe Phe 45	act tca ctt act Thr Ser Leu Thr	tat ctc tac atg Tyr Leu Tyr Met 80	gta ttt aga tat Val Phe Arg Tyr 95	gag ttc tat cta Glu Phe Tyr Leu 110
gct att Ala Ile	agt gat Ser Asp	gcg gca ac Ala Ala Th 45	tgg aaa Trp Lys 60	tgg cat Trp His 75	cca aca gt Pro Thr Va	atg gtt ga Met Val Gl
ggt agt . Gly Ser 10	gat ata Asp Ile 25	atg tta Met Leu	gct aag Ala Lys	gct ttt Ala Phe	gat aca Asp Thr 90	tta caa Leu Gln 105
ata tta Ile Leu	gat cta Asp Leu	gct ggt Ala Gly 40	gtc agc Val Ser 55	ggt ata Gly Ile	act ggt Thr Gly	gtt cca Val Pro
a tta ctg 1 Leu Leu 5	c ggt ggc a Gly Gly	g gtt aca 1 Val Thr	a gac caa y Asp Gln	att act IIle Thr 70	gata gat Ile Asp 85	tta act Leu Thr
t aaa tta y Lys Leu	t gct gct a Ala Ala 20	c tgg ctg e Trp Leu 35	a gaa aga 1 Glu Arg	t ggt tta r Gly Leu	t gtt tgg y Val Trp	t tgg cta p Trp Leu 100
atg ggt Met Gly 1	ttt gct. Phe Ala	tca ttc Ser Phe	ttt gta Phe Val 50	gta tct Val Ser 65	aga ggt Arg Gly	att gat Ile Asp

384	432	480	528	576	624	672
.,	•	•	-,	<b>-</b> ,		•
ä ä	fi e	<u>Б</u> 4 0	ta al	වා	۲ ۲	<b>ለ</b> ብ
ctt Leu	gc Al	tgg Trp 160	δ > .	aag Lys	ggt Gly	ati II
aag Lys	gaa Glu	ggt Gly	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc $_{ m G1Y}$	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta Leu
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aat Asn	gga Gly	aac Asn 220
tca Ser	gga $_{ m G1Y}$	ctt Leu 155	gaa Glu	gct tac Ala Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gct Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	cat His	aac Asn	att Ile 200	gta Val
agt Ser	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gac Asp
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga Gly	ggt Gly
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	att Ile	agt Ser
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Ty <i>r</i>	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	ggt Gly 145	tta Leu	agt Ser	att Ile	tac Tyr
					,	

Figure 28

720	753
att Ile 240	
atc Ile	
ttg Leu	
ggt Gly	
ttt Phe	
cta Leu 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
aac ctt Asn Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

PCT	US01	/14394

WO 01/8	33701	•	75/108			PCT/US01/143
48	96	144	192	240	288	336
ata tta ggt agt gct att gca ctt cca tca Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser 10	gat cta gat ata agt gat act gtt ggt gtt Asp Leu Asp Ile Ser Asp Thr Val Gly Val 25	gct ggt atg tta gcg gca act gtg ttc ttt Ala Gly Met Leu Ala Ala Thr Val Phe Phe 40	gtc agc gct gag tgg aaa act tca ctt act Val Ser Ala Glu Trp Lys Thr Ser Leu Thr 55	ggt ata gct ttt tgg cat tat ctc tat atg Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 75	act Thr	gtt cca tta caa atg gtt gag ttc tat cta Val Pro Leu Gln Met Val Glu Phe Tyr Leu 105
tta ctg Leu Leu 5	ggt ggc Gly Gly	gtt aca Val Thr	gac caa Asp Gln	att act Ile Thr 70	ata gat Ile Asp 85	tta act Leu Thr
aaa tta t Lys Leu L 5	gct gct g Ala Ala G 20	tgg ctg g Trp Leu V 35	gaa aga g Glu Arg A	ggt tta a Gly Leu I	gtt tgg a Val Trp I 8	tgg tta t Trp Leu L
τ Γa	a t A G	n e n Ei w	g A	ה ממ	η γ. Q ζ	ij (t

tca ttc Ser Phe

ttt gct gct Phe Ala Ala

ggt aaa tta Gly Lys Leu

atg Met 1

Figure 29

aga ggt gtt Arg Gly Val

att gat tgg Ile Asp Trp

tct ggt Ser Gly

gta Val 65

ttt gta ç Phe Val ( 50

384	432	480	528	576	624	672
ctt gct gct tgt aca agt gtt gct gct tca tta ttt aag aag ctt Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115	gct ggt tca tta gta atg tta ggt gct gga ttt gca ggc gaa gct A3 Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130	tta gct cct gta tta cct gct ttc att att ggt atg gct gga tgg Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 150	tac atg att tat gag cta tat atg ggt gaa ggt aag gct gct gta 52 Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 170	act gca agt cct gct gtt aac tct gca tac aac gca atg atg atg Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180	att gtt gtt gga tgg gca att tat cct gct gga tat gct gct ggt 62 Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 205	cta atg ggt ggc gaa ggt gta tac gct tca aac tta aac ctt ata 67 Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210
att Ile	cta Leu	gga G1 <u>Y</u> 145	tta Leu	agt Ser	att Ile	tac Tyr

Figure 29

720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	
cta t Leu 1 235	gct Ala
att Ile	aat Asn 250
aag Lys	tat Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	ааа Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

48	9	144	192	240	. 288	336
		•				
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr 1	aga Arg '	tat Tyr ]
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thir	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gcc Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	gac Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tct Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 30

WO 01/83701		PCT/US01/14394
	79/108	

384	432	480	528	576	624	672
ctt Leu	gct Ala	tgg Trp 160	gta Val	atg Met	ggt Gly	ata Ile
aag Lys	gaa Glu	gga Gly	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	cta Leu
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aac Asn	gga G1y	aac Asn 220
tca Ser	gga Gly	att Ile 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	tat Tyr	aac Asn	att Ile 200	gta Val
aat Asn	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tgg Trp 150	gag Glu	gct Ala	tgg Trp	gaa Glu
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga Gly	ggc Gly
gct Ala	tca Ser	cct	att Ile	agt Ser 180	gtt Val	ggt Gly
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	gga Gly 145	tta Leu	agt Ser	att Ile	tac Tyr

Figure 30

720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	
cta Leu 235	gct Ala
att. He	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat gtt Asn Val
tat Tyr 225	tgg Trp

WO 01/83701		PCT/US01/14394
•	81/108	,

48	96	144	192	240	288	336
					.,	<b>(</b> -1
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tac Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gta Val	tca Ser	ctc Leu	ttt Phe	ttc Phe 110
gcg Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	aca Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg	Gly	acg Thr	caa Gln	act Thr 70	gat Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt t Gly 1	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tct Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 31

WO 01/83701		PCT/US01/14394
	82/108	

384	432	480	528	576	624	672
•				·		
ctt Leu	gct Ala	tgg Trp 160	gta Val	atg Met	ggt, Gly	ata Ile
aag Lys	gaa Glu	gga G1Y	gct Ala 175	atg Met	gct Ala	ctc Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta Leu
tta Leu	tct Ser 140	ggt Gly	ggt Gly	aac Asn	gga Gly	aac Asn 220
tca Ser	gga Gly	att Ile 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	tat Tyr	aac Asn	att Ile 200	gta Val
agt Ser	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gaa Glu
tgt Cys	ttg Leu	gta Val	tat Tyr 165	cct Pro	gga Gly	ggc Gly
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	gtt Val	ggt Gly
gcc Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	gga G1 <u>y</u> 145	tta Leu	agt ` Ser	att Ile	tac Tyr

Figure 31

720	753
c att e Ile 240	
at Il	
ttg Leu	
ggt Gly	e agil sire i sag
.i) (i)	-
cta tti Leu Phe 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	ааа Lys
gac Asp	gtt Val 245
ctt gct g Leu Ala A	gtt gct Val Ala
ctt	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

			84/108			
48	96	144	192	240	288	336
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	gac Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa G1u	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tct Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 32

WO 01/8	33701		85/108			PCT/US01/1	
384	432	480	528	576	624	672	
ctt gct gct tgt aca aat gtt gct gct tca tta ttt aag aag ctt e Leu Ala Ala Cys Thr Asn Val Ala Ala Ser Leu Phe Lys Lys Leu 115	a gct ggt tca tta gta atg tta ggt gct gga ttt gca ggc gaa gct 1 Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130	a tta gct cct gta tgg cct gct ttc att att ggt atg gct gga tgg 7 Leu Ala Pro Val Trp Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 5	tac atg att tat gag cta tat atg ggt gaa ggt aag gct gct gta 1 Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 170	c act gca agt cct gct gtt aac tct gca tac aac gca atg atg gtg c Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Val 180	att gtt gtt gga tgg gca att tat cct gct gga tat gct gct ggt e Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 200	cta atg ggt ggc gaa ggt gta tac gct tca aac cta aac ctt ata Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210	
att Ile	cta Leu	gga Gl <u>y</u> 145	tta Leu	agt Ser	att Ile	tac Tyr	

Figure 32

720	753
c att e Ile 240	
at Il	
ttg Leu	,
ggt Gly	
ttt Phe	
cta Leu 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

			87/108			
48	96	144	192	240	288	336
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
ССА Рго 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	gac Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

figure 33

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384	432	480	228	576	624	672
ctt Leu	gct Ala	tgg Trp 160	gta Val	atg Met	ggt Gly	ata Ile
aag Lys	gaa Glu	gga G1y	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aac Asn	gga $_{ m G1y}$	aac Asn 220
tca Ser	gga G1y	att Ile 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att. Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Ty <i>r</i>	tac Tyr
gtt Val 120	tta Leu	gct Ala	tat Tyr	aac Asn	att Ile 200	gta Val
agt Ser ,	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gaa G1u
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga G1y	ggc Gly
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	gtt Val	ggt Gly
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	gga G1y 145	tta Leu	agt Ser	att Ile	tac Tyr

WO 01/83701

Figure 33

753

720

y atc att 1 Ile Ile 240	
ttg Leu	
ggt Gly	
ttt Phe	
cta Leu 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ctt Leu 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

48	96	144	192	240	 288 2	336
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	gtg Val
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	gac Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 34

D	CT	/TISO	1/1	4394
	<b>.</b>	/ U.SU	1/1	4.774

WO 01/83701		PCT/US01/14394
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384	432	480	528	576	624	672
aag aag ctt Lys Lys Leu	ggc gaa gct Gly Glu Ala	gct gga tgg Ala Gly Trp 160	gct gct gta Ala Ala Val 175	atg atg atg Met Met Met 190	gct gct ggt Ala Ala Gly	aac ctt ata Asn Leu Ile
tta ttt Leu Phe 125	ttt gca Phe Ala 140	ggt atg Gly Met	ggc aag Gly Lys	aac gca Asn Ala	gga tat Gly Tyr 205	aac tta Asn Leu 220
tca Ser	gga Gly	att Ile 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct gct Ala Ala )	ggt gct 1 Gly Ala	ttc att Phe Ile	atg ggt Met Gly 170	cct gca Pro Ala 185	tat cct Tyr Pro	tac gct Tyr Ala
agt gtt Ser Val 120	atg tta Met Leu 135	cct gct Pro Ala	cta tat Leu Tyr	gtt aac Val Asn	gca att Ala Ile 200	ggt gta Gly Val 215
tgt aca Cys Thr	tta gta Leu Val	gta tta Val Leu 150	tat gag Tyr Glu 165	cct gct Pro Ala	gga tgg Gly Trp	ggc gaa Gly Glu
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	gtt Val	ggt Gly
ctt gct Leu Ala 115	gct ggt Ala Gly 130	tta gct Leu Ala	tac atg Tyr Met	act gca Thr Ala	att gtt Ile Val 195	cta atg Leu Met 210
att Ile	cta Leu	gga Gl <u>y</u> 145	tta Leu	agt Ser	att Ile	tac Tyr

Figure 34

720	753
ttg atc att Leu Ile Ile 240	
ggt Gly	
ttt Phe	
cta Leu 235	gct Ala
att Ile	aat Asn 250
aag Lys	tct Ser
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt	gtt Val
aac Asn J	aat Asn
tat Tyr 225	tgg Trp

Figure 3.

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	. 😈	93/108	

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48	0. 0	144	192	40		36
		77		73	7	M
nt ti	1 <b>.</b>		13. 6.	<b>.</b>		
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tac Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gta Val	tca Ser	ctc Leu	ttt Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	aca Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca	caa Gln	act Thr 70	gat Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att	ata 11e 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tat Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile
					•	

Figure 35

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	0.4/1.00	

4	2	0	œ	9	<del>ct</del> i	0.7
38	433	48(	528	57(	624	672
ctt Leu	gct Ala	tgg Trp 160	gta Val	aag Lys	ggt Gly	ata Ile
aag Lys	gaa Glu	gga Gly	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta Leu
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aac Asn	gga Gly	aac Asn 220
tca Ser	gga Gly	att Ile 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	cat His	aac Asn	att Ile 200	gta Val
agt Ser	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggt Gly 215
aca Thr	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gac Asp
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga Gly	ggt Gly
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	att Ile	agt Ser
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	ggt Gly 145	tta Leu	agt Ser	att Ile	tac Tyr

Figure 35

720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	
cta Leu 235	aat gct Asn Ala 250
aag att Lys Ile	tct aat Ser Asn 250
aac Asn	tct Ser
gtt Val	gaa Glu
ttt Phe 230	aaa Lys
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

48	96	144	192	240	288	336
tca Ser	gtt Val	ttt Phe	act Thr	atg Met 80	tat Tyr	a Te
						cta Leu
cca Pro 15	ggt Gly	ttc Phe	ctt Leu	tat Tyr	aga Arg 95	tat Tyr
ctt Leu	gtt Val 30	gtg Val	tca Ser	ctc Leu	ttc Phe	ttc Phe 110
gca Ala	act Thr	act Thr 45	act Thr	tat Tyr	gta Val	gag Glu
att Ile	gat Asp	gca Ala	aaa Lys 60	cat His	aca Thr	gtt Val
gct Ala	agt Ser	gcg Ala	tgg Trp	tgg Trp 75	cca Pro	atg Met
agt Ser 10	ata Ile	tta Leu	aag Lys	ttt Phe	acc Thr 90	caa Gln
ggt Gly	gat Asp 25	atg Met	gct Ala	gct Ala	gat Asp	tta Leu 105
tta Leu	cta Leu	ggt Gly 40	agc Ser	ata Ile	ggt Gly	cca Pro
ata Ile	gat Asp	gct Ala	gtc Val 55	ggt Gly	act Thr	gtt Val
ctg Leu	ggc Gly	aca Thr	caa Gln	act Thr 70	gat Asp	act Thr
tta Leu 5	ggt Gly	gtt Val	gac Asp	att Ile	ata Ile 85	tta Leu
tta Leu	gct Ala 20	ctg Leu	aga Arg	tta Leu	tgg Trp	tta Leu 100
aaa Lys	gct Ala	tgg Trp 35	gaa Glu	ggt Gly	gtt Val	tgg Trp
ggt Gly	gct Ala	ttc Phe	gta Val 50	tct Ser	ggt Gly	gat Asp
atg Met 1	ttt Phe	tca Ser	ttt Phe	gta Val 65	aga Arg	att Ile

Figure 36

,						
384	432	480	528	576	624	672
ctt Leu	gct Ala	tgg Trp 160	gta Val	atg Met	ggt Gly	ata Ile
aag Lys	gaa Glu	gga Gly	gct Ala 175	atg Met	gct Ala	ctt Leu
aag Lys	ggc Gly	gct Ala	gct Ala	atg Met 190	gct Ala	aac Asn
ttt Phe 125	gca Ala	atg Met	aag Lys	gca Ala	tat Tyr 205	tta Leu
tta Leu	ttt Phe 140	ggt Gly	ggt Gly	aac Asn	gga Gly	aac Asn 220
tca Ser	gga Gly	att 11e 155	gaa Glu	tac Tyr	gct Ala	tca Ser
gct Ala	gct Ala	att Ile	ggt Gly 170	gca Ala	cct Pro	gct Ala
gct Ala	ggt Gly	ttc Phe	atg Met	tct Ser 185	tat Tyr	tac Tyr
gtt Val 120	tta Leu	gct Ala	tat Tyr	aac Asn	att Ile 200	gta Val
agt Ser	atg Met 135	cct Pro	cta Leu	gtt Val	gca Ala	ggc G1y 215
aca	gta Val	tta Leu 150	gag Glu	gct Ala	tgg Trp	gaa Glu
tgt Cys	tta Leu	gta Val	tat Tyr 165	cct Pro	gga Gly	ggc
gct Ala	tca Ser	cct Pro	att Ile	agt Ser 180	gtt Val	ggt Gly
gct Ala 115	ggt Gly	gct Ala	atg Met	gca Ala	gtt Val 195	atg Met
ctt Leu	gct Ala 130	tta Leu	tac Tyr	act Thr	att Ile	cta Leu 210
att Ile	cta Leu	gga G1 <u>y</u> 145	cta Leu	agt Ser	att Ile	tac Tyr
. •						

Figure 36

720	753
ttt ggt ttg atc att Phe Gly Leu Ile Ile 240	· .
cta Leu 235	t gct n Ala 0
aag att Lys Ile	tct aa Ser-As 25
t aac 1 Asn	aa tct lu Ser
ttt gtt Phe Val 230	aaa ga Lys Gl
gac Asp	gtt Val 245
gct Ala	gct Ala
ctt Leu	gtt Val
aac Asn	aat Asn
tat Tyr 225	tgg Trp

Figure 3

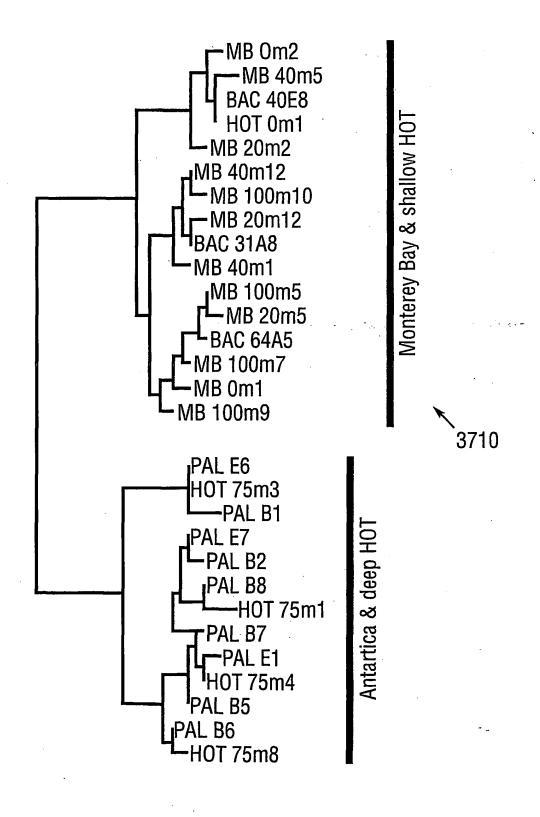
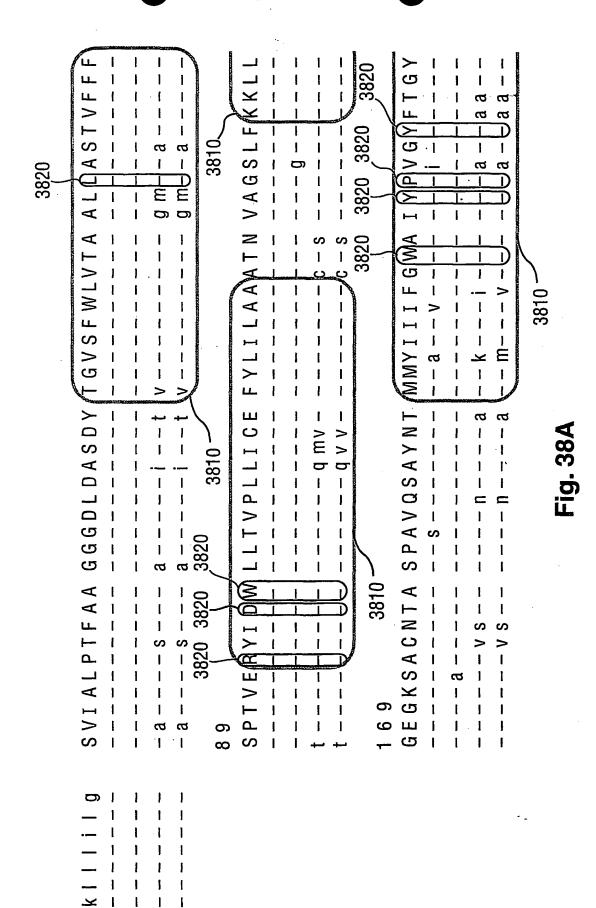
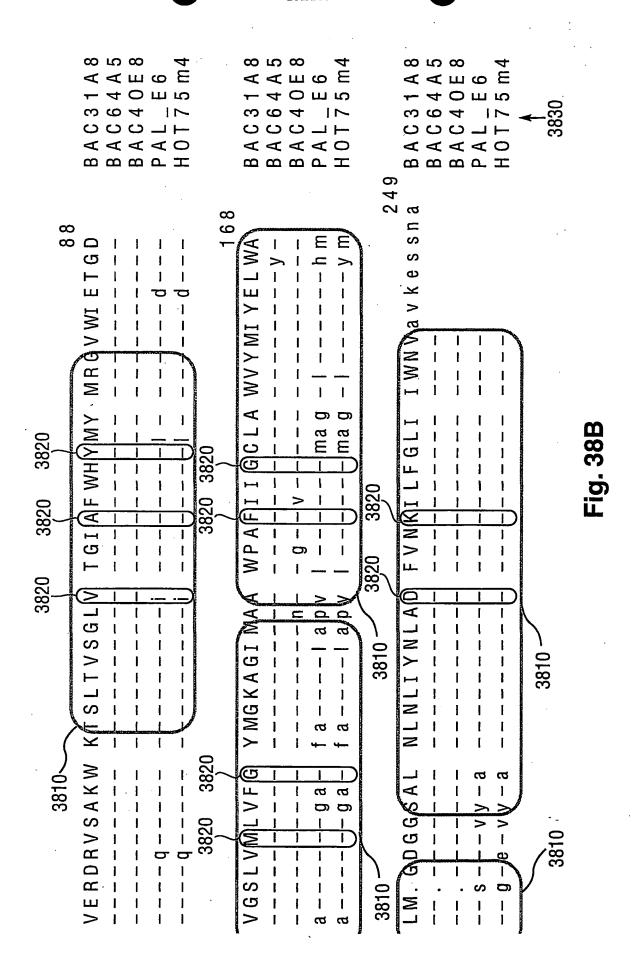


Fig. 37





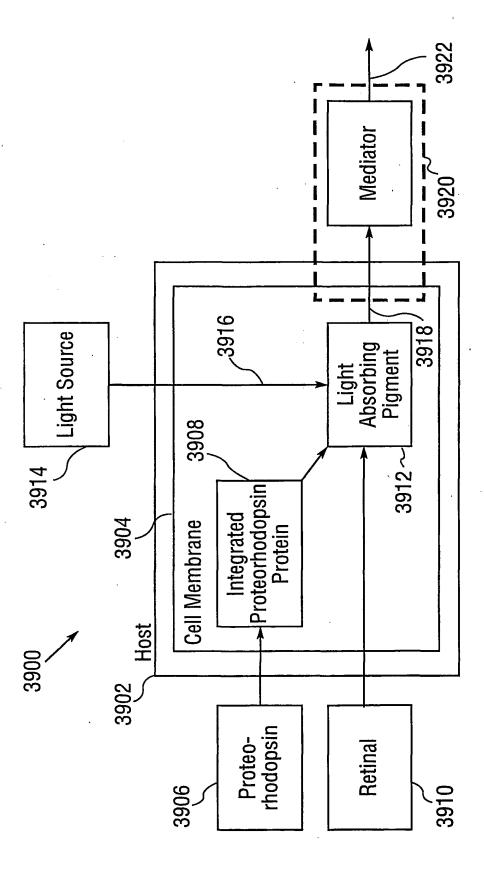
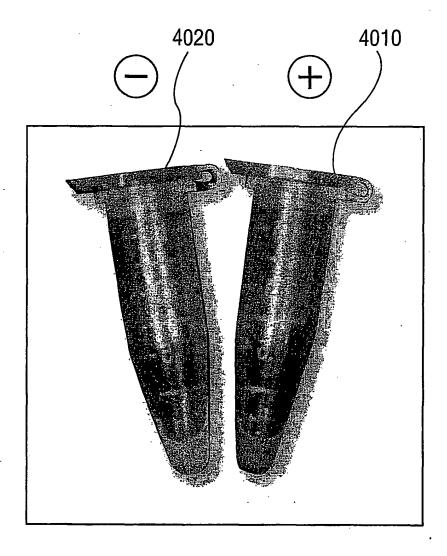


Fig. 39



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Fig. 40

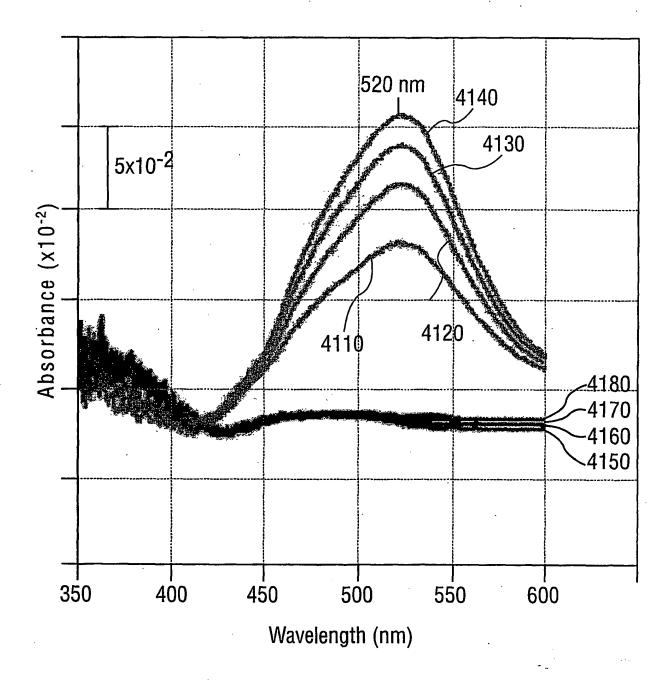


Fig. 41 BEST AVAILABLE COPY

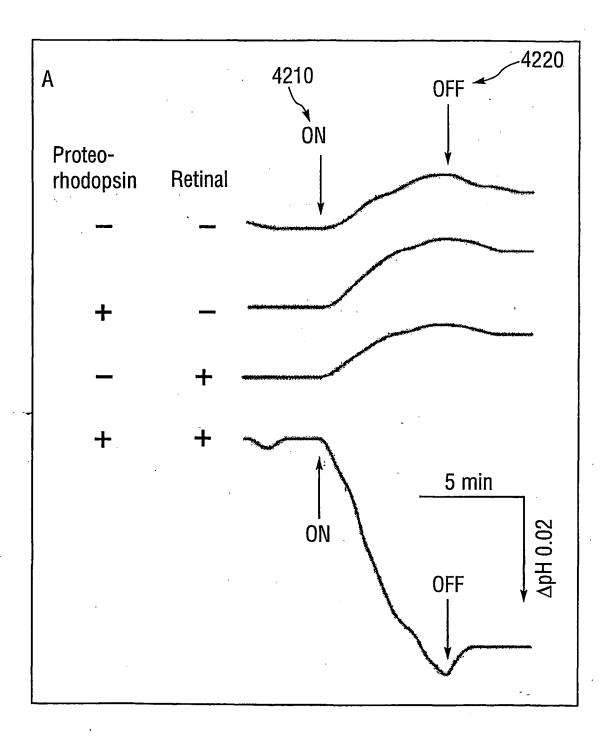


Fig. 42

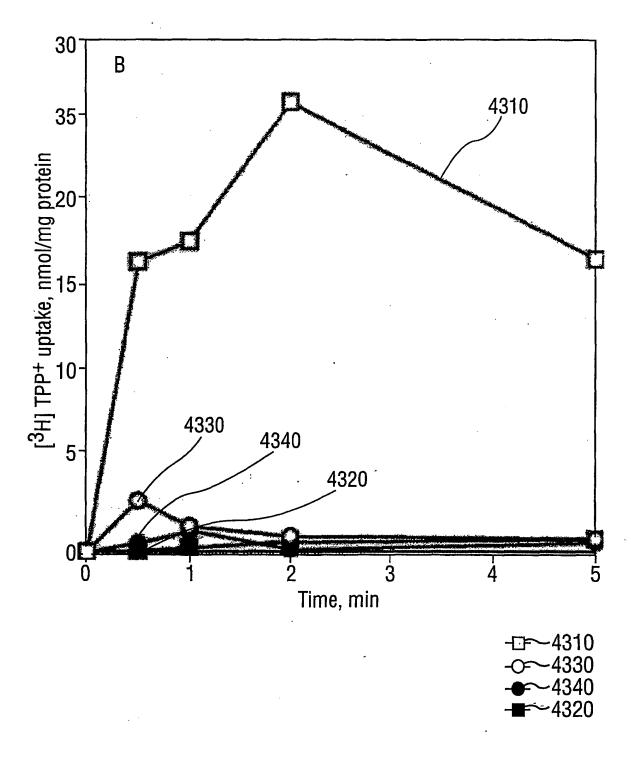


Fig. 43 BEST AVAILABLE COPY

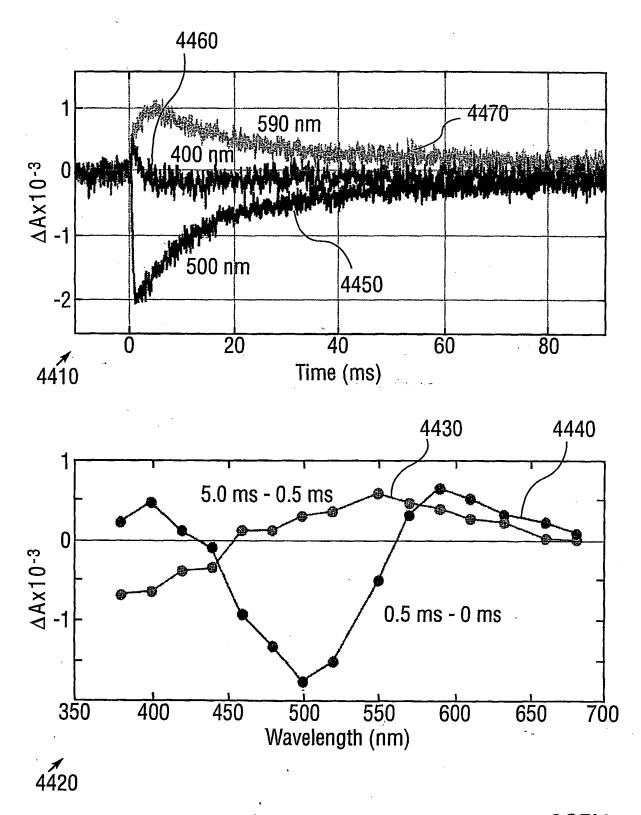
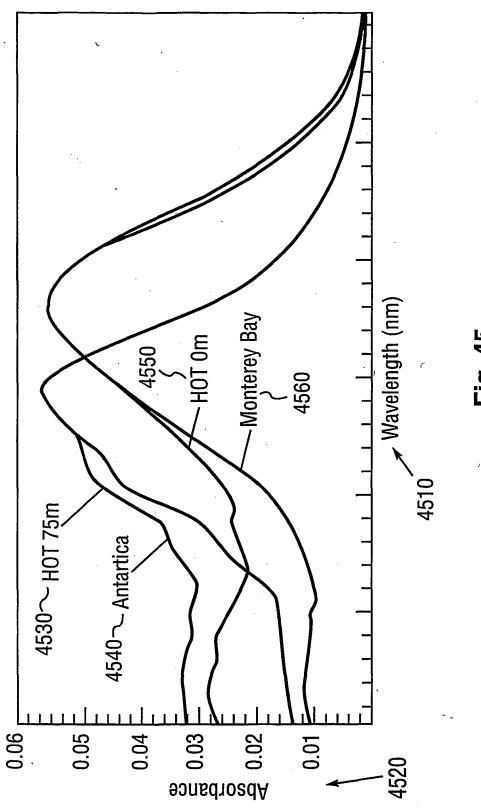


Fig. 44 BEST AVAILABLE COPY



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## SEQUENCE LISTING

<110> MBARI DeLong, Edward Beja, Oded
<120> Light-driven energy generation using proteorhodopsin
<130> MBA-101
<150> 60/201,602 <151> 2000-05-03
<160> 65
<170> PatentIn version 3.0
<pre>&lt;210&gt; 1 &lt;211&gt; 105184 &lt;212&gt; DNA &lt;213&gt; Naturally occurring gamma proteobacterium  &lt;220&gt; &lt;221&gt; CDS(complement) &lt;222&gt; (50866)(51615) &lt;223&gt; light-driven proton pump; has the properties of a light-driven proton pump when expressed with retinal in Escherichia col</pre>
<pre>&lt;300&gt; &lt;301&gt; Beja,O., Aravind,L., Koonin,E.V., Suzuki,M.T., Hadd,A.,Nguyen,L.P., Jovanovich,S.B., Gates,C.M., Feldman,R.A., DeLong,E.F &lt;302&gt; Bacterial rhodopsin: evidence for a new type of phototrophy in the sea &lt;303&gt; Science &lt;304&gt; 289 &lt;305&gt; 5486 &lt;306&gt; 1902-1906 &lt;307&gt; 2000-09-15 &lt;308&gt; AF279106 &lt;309&gt; 2000-06-15 &lt;313&gt; (50866)(51615)</pre>
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tcttctagat acagacatga gagttcttga ttccgctgag tcaagaaacc tttgcgagtt 180
tgaaggaaaa gctttactag ttgtgaatgt tgcaagtaga tgtggttaca cttatcaata 240





















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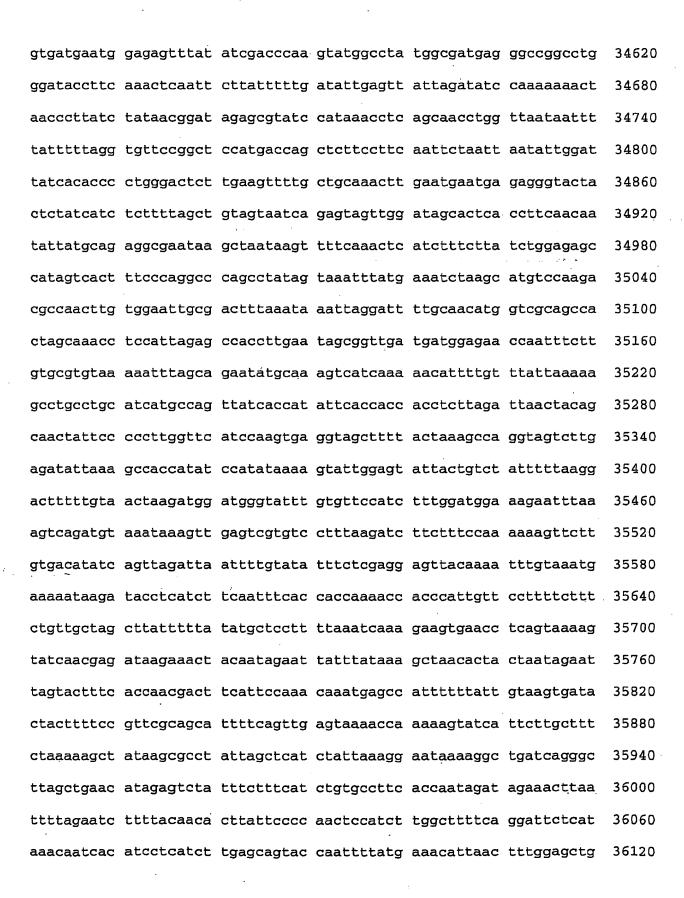
















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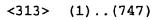
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										gaa Glu							528
									-	tac Tyr							576
						_				gta Val							624
										aac Asn					tat- Tyr		672
gac	ctt	gct	gac	ttt	gtt	aac	aag	att	cta	ttt	ggt	tta	att	ata	tgg		720

Asp Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 aat gtt gct gtt aaa gaa tct tct aat gct 750 Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 <210> 9 <211> 250 <212> PRT <213> Naturally occurring gamma proteobacterium <400> 9 Met Gly Lys Leu Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45 Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60 Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 70 75 Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95 Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110 Ile Leu Ala Ala Ala Thr Asn Val Ala Ala Gly Leu Phe Lys Lys Leu 115 120 125 Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala-130 135

Gly Ile Met Asn Ala Trp Gly Ala Phe Val Ile Gly Cys Leu Ala Trp

145 150 155 160 Val Tyr Met Ile Tyr Glu Leu Trp Ala Gly Glu Gly Lys Ala Ala Cys 165 Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Tyr 180 185 190 Ile Ile Ile Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly 195 200 205 Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220 \* Asp Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 Control of State (1987) Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 <210> 10 <211> 750 <212> DNA <213> Naturally occurring gamma proteobacterium <220> <221> CDS <222> (1)..(750) <223> proteorhodpsin variant from clone EBAC41 <400> 10 atg ggt aaa tta tta ctg ata tta ggt agt gtt att gca ctt cct aca 48 Met Gly Lys Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr ttt gct gca ggt ggt ggt gac ctt gat gct agt gat tac act ggt gtt 96 Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 tet ttt tgg tta get act get get tta tta gea tet act gta ttt tte 144 Ser Phe Trp Leu Ala Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 45 ttt gtt gaa aga gat aga gtt tct gca aaa tgg aaa aca tca tta act 192

Phe	Val 50	Glu	Arg	Asp	Arg	Val 55	Ser	Ala	Lys	Trp	Lys 60	Thr	Ser	Leu	Thr		
													atg Met			•	240
													ttt Phe				288
													ttc Phe 110				336
													aag Lys				384
													ggt Gly				432
				Ala									tta Leu				480
									. — —	_			tct Ser	_	_		528
		Ala											atg Met 190				576
													ttc Phe				624
	-					-		_					ctt Leu				672
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<211> 250

<212> PRT

<213> Naturally occurring gamma proteobacterium

<400> 11

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Ser Phe Trp Leu Ala Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Trp Ala Gly Glu Gly Lys Ser Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Tyr

65

180 185 190 Ile Ile Ile Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly 195 200 Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220 Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240 Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 <210> 12 <211> 750 <212> DNA <213> Naturally occurring gamma proteobacterium <220> <221> CDS. <222> (1)..(750)<223> Proteorhodopsin variant from clone EBAC64 <400> 12 atg ggt aaa tta tta ctg ata tta ggt agt gtt att gca ctt cct aca 48 Met Gly Lys Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr 10 ttt get gea ggt gge ggt gae ett gat get agt gat tae act ggt gtt 96 Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 tet ttt tgg tta gtt aca get get eta tta gea tet act gta ttt tte 144 Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 ttt gtt gaa aga gat aga gtt tct gca aaa tgg aaa aca tca tta act 192 Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60 gta tot ggt ott gtt act ggt att gct tto tgg cat tac atg tac atg-240 Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met

aga gga gta tgg att gaa act ggt gat tcg cct act gta ttt aga tac

75

80

288

70

Arg	Gly	Val	Trp	Ile 85	Glu	Thr	Gly	Asp	Ser 90	Pro	Thr	Val	Phe	Arg 95	Tyr	
	gat Asp										_	-				336
	ctt ·Leu															384
	gtt Val 130															432
	att Ile															480
	tac Tyr															528
	act Thr								_					_		576
	ata Ile	_				_										624
	cta Leu 210	_		_	-			. —								672
	ctt Leu															720
	gtt Val															750

<210> 13

<211> 250

<212> PRT

<213> Naturally occurring gamma proteobacterium

<400> 13

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Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr

210 215 220

Asn	Leu	Ala	Asp	Phe	Val Asn	Lys	Ile	Leu	Phe	Gly	Leu	Ile	Ile	Trp
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Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 14 <211> 750

<212> DNA

<213> Naturally occurring gamma proteobacterium

<220>

<221> CDS

<222> (1)..(750)

<223> Proteorhodopsin variant from pcr clone HOT01m: GenBank# AF349978

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	gct Ala	_	 		_		_	_	_	_			 _		96
	ttt Phe		_		_	_			_			_			144
	gtt Val 50			-	_		_								192
	tcg Ser														240
	Gl <sup>y</sup> aaa														288
	gat Asp														336

att ctt gct gct Ile Leu Ala Ala 115	gca aca aat Ala Thr Asn	gtt gct gct Val Ala Ala 120	ggc ctg ttt Gly Leu Phe 125	aag aaa Lys Lys	tta 384 Leu
ttg gtt ggt tct Leu Val Gly Ser 130					
gga att atg aac Gly Ile Met Asn 145	gct tgg ggt Ala Trp Gly 150	gca ttc gtt Ala Phe Val	att ggg tgt Ile Gly Cys 155	tta gct Leu Ala	tgg 480 Trp . 160
gta tac atg att Val Tyr Met Ile					
aat act gca agt Asn Thr Ala Ser 180					
ata atc atc ttt Ile Ile Ile Phe 195	ggt tgg gca Gly Trp Ala	att tat cct Ile Tyr Pro 200	gta ggt tat Val Gly Tyr 205	ttc aca Phe Thr	ggt 624 Gly
tac cta atg ggt Tyr Leu Met Gly 210					
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Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 30

Ser	Phe	Trp 35	Leu	Val	Thr	Ala	Ala 40	Leu	Leu	Ala	Ser	Thr 45	Val	Phe	Phe
Phe	Val 50	Glu	Arg	Asp	Arg	Val 55	Ser	Ala	Lуз	Trp	Lys 60	Thr	Ser	Leu	Thr
Val 65	Ser	Gly	Leu	Val	Thr 70		Ile	Ala		Trp 75	His	Tyr	Met	Tyr	Met 80
Arg	Gly	Val	Trp	Ile 85	Glu	Thr	Gly	Asp	Ser 90	Pro	Thr	Val	Phe	Arg 95	Туг
Ile	Asp	Trp	Leu 100	Leu	Thr	Val	Pro	Leu 105	Leu	Ile	Cys	Glu	Phe 110	Tyr	Leu
Ile	Leu	Ala 115	Ala	Ala	Thr	Asn	Val 120		Ala	Gly	Leu	Phe. 125	Lys	Lys.	Leu
Leu	Val 130	Gly	Ser	Leu	Val	Met 135	Leu	Val	Phe	Gly	Tyr 140	Met	Gly	Glu	Ala
Gly 145		Met	Asn	Ala	Trp 150	Gly	Ala	Phe	Val	Ile 155	Gly	Суз	Leu	Ala	Trp 160
Val	Tyr	Met	Ile	Туг 165	Glu	Lęu	Trp	Ala	Gly 170	Glu	Gly	Lys	Ala	Ala 175	Cys
Asn	Thr	Ala	Ser 180	Pro	Ala	Val	Gln	Ser 185	Ala	Tyr	Asn	Thr	Met 190	Met	Tyr
Ile	Ile	Ile 195	Phe	Gly	Trp	Ala	Ile 200	Tyr	Pro	Val	Gly	Tyr 205	Phe	Thr	Gly
Tyr	Leu 210	Met	Gly	Asp	Gly	Gly 215	Ser	Ala	Leu	Asn	Leu 220	Asn	Leu	Ile	Tyr
Asn 225	Leu	Ala	Asp	Phe	Val 230	Asn	·Lys	Ile	Leu	Phe 235	Gly	Leu	Ile	Ile	Trp 240

<210> 16

## Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<21: <21: <21:	l> ' 2> 1	753 DNA Natu	cally	y oco	curr	ing (	yamma	a pri	toeol	oacte	eriu	n .		. •			
<220 <220 <220 <220	1> ( 2>	CDS (1). Prote			sin v	varia	ant :	Érom	pcr	clo	ne HO	OT751	n1: (	GenBa	ank#1	\F349	979
<40	) > ·	16													•	٠	·
atg	ggt	aaa Lys															48
		gct Ala															96
		tgg Trp 35	_	_		_		_			_						144
		gaa Glu															192
		ggt Gly															240
		gtt Val															288
		tgg Trp															336
		gct Ala 115															384
cta Leu	gct Ala 130	ggt Gly	tca Ser	tta Leu	gta Val	atg Met 135	tta Leu	ggt Gly	gct Ala	gga Gly	ttt Phe 140	gca Ala	ggc Gly	gaa Glu	gct Ala		432

			•			
	cct gta tta Pro Val Leu 150	Pro Ala Ph				480
	g att tat gag : Ile Tyr Glu 165	,				528
	a agt cct gct a Ser Pro Ala 180		r Ala Tyr A			576
	gtt gga tgg Val Gly Trp					624
-	g ggt ggc gaa : Gly Gly Glu		r Ala Ser A			672
	gcc gac ctt Ala Asp Leu 230	Val Asn Ly	_			720
	gct gtt aaa Ala Val Lys 245		_			753
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	rarry occurr	rng ganda p	rcoeopacter	. L cuit		•
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Met Gly Lys 1	: Leu Leu Leu 5	Ile Leu Gl	y Ser Ala I 10		Pro Ser 15	
Phe Ala Ala	Ala Gly Gly 20	Asp Leu As	p Ile Ser A	asp Thr Val	Gly Val	
Ser Phe Trp	Leu Val Thr	Ala Gly Me	t Leu Ala A	ala Thr Val	Phe Phe	
Phe Val Glu 50	ı Arg Asp Gln	Val Ser Al 55	_	ys Thr Ser 0	Leu Ala	

Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met - 70 75. Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125 Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 150 155 Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 175 Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180 185 Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195 200 Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220 Tyr Asn Leu Ala Asp Leu Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 230 225 235 240

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala

245

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<211> 753

<212> DNA

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ttt gct gct ggt ggc gat cta gat ata agt gat act gtt ggt gtt 96 Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20 25 30
tca ttc tgg ctg gtt aca gct ggt atg tta gcg gca act gta ttc ttt  Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe  35  40  45
ttt gta gaa aga gac caa gtc agc gct aag tgg aaa act tca ctt act 192 Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60
gta tct ggt tta att act ggt ata gct ttt tgg cat tat ctc tac atg Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 65 70 75 80
aga ggt gtt tgg ata gat act ggt gat aca cca aca gta ttt aga tat 288 Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 95
att gat tgg tta tta act gtt cca tta caa atg gtt gag ttc tat cta 336 Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 110
att ctt gct gct tgt aca agt gtt gct gct tca tta ttt aag aag ctt 384  Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125
cta gct ggt tca tta gta atg tta ggt gct gga ttt gca ggc gaa gct Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 140
ggt tta gct cct gta tta cct gct ttc att att ggt atg gct gga tgg Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Tro 145 150 155 160
tta tac atg att tat gag cta cat atg ggt gaa ggt aag gct gct gta 528 Leu Tyr Met Ile Tyr Glu Leu His Met Gly Glu Gly Lys Ala Ala Val

		•	
	165	170	175
	Pro Ala Val Asn Ser	gca tac aac gca atg Ala Tyr Asn Ala Met 190	
		cct gct gga tat gct Pro Ala Gly Tyr Ala 205	
		gct tca aac tta aac Ala Ser Asn Leu Asn 220	
		att cta ttt ggt ttg Ile Leu Phe Gly Leu 235	
	gtt aaa gaa tct tct Val Lys Glu Ser Ser 245		753
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•	y occurring gamma pr	oteobacterium	·
•	y occurring gamma pr	oteobacterium	
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100

110

Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 135 Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 150 155 Leu Tyr Met Ile Tyr Glu Leu His Met Gly Glu Gly Lys Ala Ala Val 165 170 Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Lys 180 Ile Ile Val Ile Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195 200 205 Tyr Leu Met Ser Gly Asp Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220 Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235 Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala <210> 20 <211> 753 <212> DNA <213> Naturally occurring gamma proteobacterium <220> <221> CDS <222> (1)..(753)<223> Proteorhodopsin variant from pcr clone HOT75m4; GenBank #AF349981

Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu

105

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		gct Ala															96
tca Ser	ttc Phe	tgg Trp 35	ctg Leu	gtt Val	aca Thr	gct Ala	ggt Gly 40	atg Met	tta Leu	gcg Ala	gca Ala	act Thr 45	gtg Val	ttc Phe	ttt Phe	·	144
ttt Phe	gta Val 50	gaa Glu	aga Arg	gac Asp	caa Gln	gtc Val 55	agc Ser	gct Ala	aag Lys	tgg Trp	aaa Lys 60	act Thr	tca Ser	ctt Leu	act Thr		192
gta Val 65	tct Ser	ggt Gly	tta Leu	att Ile	act Thr 70	ggt Gly	ata Ile	gct Ala	ttt Phe	tgg Trp 75	cat His	tat Tyr	ctc Leu	tat Tyr	atg Met 80		240
		gtt Val													tat Tyr		288
att Ile	gat Asp	tgg Trp	tta Leu 100	tta Leu	act Thr	gtt Val	cca Pro	tta Leu 105	caa Gln	gtg Val	gtt Val	gag Glu	ttc Phe 110	tat Tyr	cta Leu		336
		gct Ala 115															384
cta Leu	gct Ala 130	ggt Gly	tca Ser	tťa Leu	gta Val	atg Met 135	tta Leu	ggt Gly	gct Ala	gga Gly	ttt Phe 140	gca Ala	ggc	gaa Glu	gct Ala	·	432
gga Gly 145	tta Leu	gct Ala	cct Pro	gta Val	tta Leu 150	cct Pro	gct Ala	ttc Phe	att Ile	att Ile 155	ggt Gly	atg Met	gct Ala	gga Gly	tgg Trp 160		480
		atg Met														•	528
		gca Ala															576
att Ile	att Ile	gtt Val	gtt Val	gga Gly	tgg Trp	gca Ala	att Ile	tat Tyr	cct Pro	gct Ala	gga Gly	tat Tyr	gct Ala	gct Ala	ggt Gly	ı	624

195		200	205		
	Gly Gly Glu (		gct tca aac tta Ma Ser Asn Leu 220		
			att cta ttt ggt Ile Leu Phe Gly 235		
	gct gtt aaa g Ala Val Lys G 245	Glu Ser Ser A		<sup>7</sup> 753	
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Met Gly Lys 1	Leu Leu Leu 1 5		Ser Ala Ile Ala .0	Leu Pro Ser 15	
Phe Ala Ala	Ala Gly Gly A	Asp Leu Asp I 25	le Ser Asp Thr	Val Gly Val	
Ser Phe Trp	Leu Val Thr A	Ala Gly Met L 40	eu Ala Ala Thr 45	Val Phe Phe	
Phe Val Glu 50		Mal Ser Ala L	ys Trp Lys Thr 60	Ser Leu Thr	
Val Ser Gly 65	Leu Ile Thr 0 70	Sly Ile Ala P	the Trp His Tyr 75	Leu Tyr Met 80	
Arg Gly Val	Trp Ile Asp 7		hr Pro Thr Val	Phe Arg Tyr 95	
Ile Asp Trp	Leu Leu Thr V	al Pro Leu G 105	ln Val Val Glu	Phe Tyr Leu 110	
Ile Leu Ala 115	Ala Cys Thr S	Ser Val Ala A 120	la Ser Leu Phe 125	Lys Lys Leu	

Leu	Ala 130	Gly	Ser	Leu	Val	Met 135	Leu	Gly	Ala	Gly	Phe 140	Ala	Gly	Glu	Ala	•
Gly 145	Leu	Ala	Pro	Val	Leu 150	Pro	Ala	Phe	Ile	Ile 155	Gly	Met	Ala	Gly	Trp 160	
Leu	Tyr	Met	Ile	Туг 165	Glu	Leu	Tyr	Met	Gly 170	Glu	Gly	Lys	Ala	Ala 175	Val	
Ser	Thr	Ala	Ser 180	Pro	Ala	Val	Asn	Ser 185	Ala	Tyr	Asn	Ala	Met 190	Met	Met	-
Ile	Ile	Val 195	Val	Gly	Trp	Ala	Ile 200	Tyr	Pro	Ala	Gly	Tyr 205	Ala	Ala	Gly	
Tyr	Leu 210		Gly	Gly	Glu	Gly 215		Tyr	Ala	Ser	Asn 220	Leu	Asn	Leu	Ile	
Tyr 225	Asn	Leu	Ala	Asp	Phe 230	Val	Asn	Lys	Ile	Leu 235	Phe	Gly	Leu	Ile	Ile 240	
Trp	Asn	Val	Ala	Val 245	Lys	Glu	Ser	Ser	Asn 250	Ala						
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<220 <221 <222 <223	L> ( ?> (	CDS (1) Prote	•	•	sin v	varia	int f	rom	pcr	clor	ıe HC	) <b>T</b> 75n	n8: (	enBa	nk#AF3499	82
- 4 2 -	\				-				•			•			•	
<400 atg		22 aaa	tta	tta	ctg	ata	tta	ggt	agt	gct	att	gca	ctt	cca	tca -	48
				Leu 5												
ttt	gct	gct	gct	ggt	ggc	gat	cta	gat	ata	agt	gat	act	gtt	ggt	gtt	96

	Phe	Ala	Ala	Ala 20	Gly	Gly	Asp	Leu	Asp 25	Ile	Ser	Asp	Thr	Val 30	Gly	Val		
									atg Met									144
									gct Ala							act Thr		192
									gct Ala							atg Met 80		240
									gat Asp									288
									tta Leu 105		_	_						336
									gct Ala								•	384
									ggt Gly									432
									ttc Phe									480
,									atg Met									528
									tct Ser 185	Ala								576
									tat Tyr									624
									tac Tyr									672
	tat	aac	ctt	gcc	gac	ctt	gtt	aac	aag	att	cta	ttt	ggt	ttg	atc	att		720

Tyr Asn Leu Ala Asp Leu Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 230 235 240 tgg aat gtt gct gtt aaa gaa tct tct aat gct 753 Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250 <210> 23 <211> 251 <212> PRT <213> Naturally occurring gamma proteobacterium <400> 23 Met Gly Lys Leu Leu Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20 . 25 30 Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe 35 40 45 Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 60 Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 70 Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 95 Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 Ile Leu Ala Ala Cys Thr Asn Val Ala Ala Ser Leu Phe Lys Lys Leu 115 Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 140 Gly Leu Ala Pro Val Trp Pro Ala Phe Ile Ile Gly Met Ala Gly Trp

145 150 155 160 Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Val 180 185 Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195 200 205 Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 215 Tyr Asn Leu Ala Asp Leu Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 230 235 Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250 <210> 24 <211> 750 <212> <213> Naturally occurring gamma proteobacterium <220> <221> CDS <222> (1)..(750)<223> Proteorhodopsin variant from pcr clone MBOml: GenBank#AF349983 <400> 24 atg ggt aaa tta tta ctg ata tta ggt agt gtt att gca ctt cct aca 48 Met Gly Lys Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr ttt gct gca ggt ggt ggt gac ctt gat gct agt gat tac act ggt gtt 96 Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 tet ttt tgg tta gtt act get get eta tta gea tet act gta ttt tte 144 Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45 ttt gtt gaa aga gat aga gtt tct gca aaa tgg aaa aca tca tta act 192

Phe	Val 50	Glu	Arg	Asp	Arg	Val 55	Ser	Ala	Lys	Trp	Lys 60	Thr	Ser	Leu	Thr		
					act Thr 70												240
					gag Glu												288
					aca Thr												336
					aca Thr												384
					gtt Val												432
					tgg Trp 150												480
-		_			gaa Glu			_		_				_	-		528
					tcg Ser												576
					tgg Trp										ggt Gly	•	62 <b>4</b>
					ggt Gly										tat Tyr		672
					gtt Val 230						Gly					·	720
	_				gaa Glu				-		•						750

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Asn Thr Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala

Val Tyr Met Ile Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys

165

170

180 185 190

Ile Ile Val Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly
195 200 205

PCT/US01/14394

Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 26 <211> 750

<212> DNA

<213> Naturally ocurring gamma proteobacterium

<220>

<221> CDS

<222> (1)..(750)

<223> Proteorhodopsin variant from pcr clone MB0m2

<400> 26

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Met Gly Lys Leu Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr
1 5 10 15

ttt gct gca ggt ggt ggc ctt gat gct agt gat tac act ggt gtt 96
Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val
20 25 30

tct ttt tgg tta gtt act gct gct tta tta gca tct act gta ttt ttc

144
Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe

ttt gtt gaa aga gat aga gtt tct gca aaa tgg aaa aca tca tta act

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr

50 55 60

gta tct ggt ctt gtt act ggt att gct ttc tgg cat tac atg tac atg
Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met
65 70 75 80

aga ggg gta tgg att gaa act ggt gat tcg cca act gta ttt aga tac 288

Arg	Gly	Val	Trp	I1e 85	Glu	Thr	G1y	_	Ser 90	Pro	Thr	Val	Phe	Arg 95	Tyr		
	_											gaa Glu			tta Leu		336
												ttt Phe 125			tta Leu	-	384
												atg Met					432
												tgt Cys					480
												aaa Lys					528
aat Asn	aca Thr	gca Ala	agt Ser 180	cct Pro	gct Ala	gtt Val	cag Gln	tca Ser 185	gct Ala	tac Tyr	aac Asn	aca Thr	atg Met 190	atg Met	atg Met		576
												tat Tyr 205				· .	624
												aac Asn					672
												tta Leu					720
				aaa Lys 245											-		750
																•	(4)

<210> 27

<211> 250

<212> PRT

<213> Naturally ocurring gamma proteobacterium

<400> 27

Met Gly Lys Leu Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr

1 5 10 15

Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 30

Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Ala Thr Asn Val Ala Ala Gly Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Asn Ala Trp Gly Ala Phe Val Ile Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Trp Leu Gly Glu Gly Lys Ala Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Met 180 185 190

Ile Ile Ile Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly
195 200 205

Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr

288

210 215 . 220

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 28 <211> 750 <212> DNA

85

<213> Naturally occuring gamma proteobacterium

<220>

<221> CDS

<222> (1)..(750)

<223> Proteorhodopsin variant from pcr clone MB20m2; GenBank #AF349985

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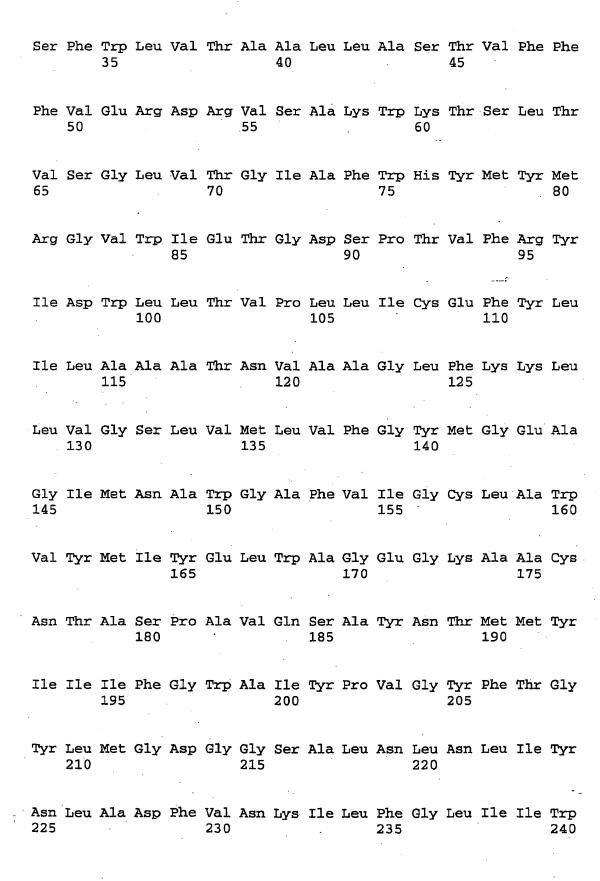
att gat tgg tta cta aca gtt cct cta tta ata tgt gaa ttc tac tta 336
Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu
100 105 110

90

aga ggg gta tgg att gaa act ggt gat tcg cca act gta ttt aga tac

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr

•														
att ctt Ile Leu														384
ttg gtt Leu Val 130														432
gga att Gly Ile 145														480
gta tac Val Tyr						-				_	-	-	_	528
aat act Asn Thr		Pro												576
ata atc Ile Ile														624
tac cta Tyr Leu 210		-				_								672
aac ctt Asn Leu 225			_		_									720
aat gtt Asn Val			_				-							750
<211> 2 <212> E	9 250 PRT Taturall	у осс	curir	ng ga	amma	prot	:eoba	icter	cium					<i>,</i>
<400> 2	!9	·												,
Met Gly	Lys Leu	Leu 5	Leu	Ile	Leu	Gly	Ser 10	Val	Ile	Ala	Leu	Pro 15	Thr	
Phe Ala	Ala Gly 20	Gly	Gly ·	Asp	Leu	Asp 25	Ala	Ser	Asp	Tyr	Thr 30	Gly	Val	



<21: <21: <21: <22: <22: <22: <22:	1> 2> 3> 0> 1> 2>	30 750 DNA Natur CDS (1).	. (750	0)				_				· .	5; G	enBar	nk#AI	r349986
<40	, ) <b>&gt;</b>	30						·				-				
atg	ggt	aaa Lys														48
		gca Ala														96
		tgg Trp 35		_		_	_			_			_			144
		gaa Glu														192
		ggt Gly														240
		gta Val														288
		tgg Trp												Tyr		336
		gct Ala 115														384
cta Leu	gtt Val 130	ggt Gly	tct Ser	ctt Leu	gtt Val	atg Met 135	ctt Leu	gtg Val	ttt Phe	ggt Gly	tac Tyr 140	atg Met	ggt Gly	gaa Glu	gca Ala	432

caa att ato Gln Ile Met 145			-					480
gta tac ato Val Tyr Met		Glu Leu				Ser A		528
aat act gca Asn Thr Ala						_		576
atc ata gto Ile Ile Val 195	. Phe Gly							624
tac cta ato Tyr Leu Met 210			_					672
aac ctt gct Asn Leu Ala 225	_	_						720
aat gtt gct Asn Val Ala	_	Glu Ser		_	-			750
<210> 31 <211> 250 <212> PRT <213> Nati	rally oc	curring (	gamma pro	oteobact	erium			
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Met Gly Lys	Leu Leu 5	Leu Ile	Leu Gly	Ser Val	Ile Ala	Leu P		
Phe Ala Ala	Gly Gly 20	Gly Asp	Leu Asp 25	Ala Ser	Asp Tyr	Thr G	ly Val	
Ser Phe Trp 35	Leu Val	Thr Ala	Ala Leu 40	Leu Ala	Ser Thr 45	Val P	he Phe	
Phe Val Glu					•			

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 70 75 Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 120 125 Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 Gln Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 Val Tyr Met Ile Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys 165 170 Asn Thr Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala 180 185 190 Ile Ile Val Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly 195 200 205 Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Leu Gly Leu Ile Ile Trp 230 235

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 32

<211> 750

<212> DNA

<213> Naturally occurring gamma proteobacterium

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atg		32 aaa Lys			_					-		-					48
		gca Ala															96
		tgg Trp 35		-		_	_			_			_				L44
	_	gaa Glu	_	_	_	_		_									Ĺ92
-		ggt Gly		_				_					_		_	. 2	240
		gta Val						-	_			_					. 88
	-	tgg Trp				_					_	_					36
		gct Ala 115					-						_			3	884
Leu		ggt Gly														4	132
		atg Met														4	180
		atg Met														5	28

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 Ile Leu Ala Ala Ala Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 135 Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 Val Tyr Met Ile Tyr Glu Leu Trp Ala Gly Glu Gly Lys Ser Ala Cys 165 170 Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Tyr 180 185 Ile Ile Ile Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly 200 Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 34 <211> 750 <212> DNA <213> Naturally occurring gamma proteobacterium

<220> <221> CDS

<222> (1)..(750)

<223> Proteorhodopsin variant from pcr clone MB40m1; GenBank #AF349988

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				ggt Gly							96
				aca Thr						1	144
				aga Arg						3	192
				act Thr 70						2	240
				gaa Glu						2	288
				aca Thr						3	336
				act Thr						3	384
				gtt Val						4	132
				tgg Trp 150						· <b>4</b>	180
				gaa Glu						5	528
				gct Ala						5	576
				tgg Trp						6	24

										ctt Leu							672	
			_	_		-				cta Leu							720	۱.
							tct Ser										750	•
	<210 <211 <212 <213	L> 2 2> 1		cally	/ occ	curri	ing g	gamma	a pro	oteok	Dacte	eriu	n					
	<400	)> 3	35				·				,							
	Met 1	Gly	Lys	Leu	Leu 5	Leu	Ile	Ile	Gly	Ser 10	Val	Ile	Ala	Leu	Pro 15	Thr		
,	Phe	Ala	Ala	Gly 20	Gly	Gly	Asp	Leu	Asp 25	Ala	Ser	Asp	Tyr	Thr 30	Gly	Val		
	Ser	Phe	Trp 35	Leu	Val	Thr	Ala	Ala 40	Leu	Leu	Ala	Ser	Thr 45	Val	Phe	Phe	,	
	Phe	Val 50	Glu	Arg	Asp	Arg	Va1 55	Ser	Ala	Lys	Trp	Lys 60	Thr	Ser	Leu	Thr		•
	Val 65	Ser	Gly	Leu	Val	Thr 70	Gly	Ile	Ala	Phe	Trp 75		-	Met	_	Met 80		
	Arg	Gly	Val	Trp	Ile 85	Glu	Thr	Gly	Asp	Ser 90	Pro	Thr	Val	Phe	Arg 95	Tyr		
	Ile	Asp	Trp	Leu 100	Leu	Thr	Val	Pro	Leu 105	Leu	Ile	Cys	Glu	Phe 110	Tyr	Leu		
	Ile	Leu	Ala 115	Ala	Ala	Thr	Asn	Val 120	Ala	Gly	Ser	Leu	Phe 125	Lys	Lys	Leu		

Leu	Val 130	Gly	Ser	Leu	Val	Met 135	Leu	Val	Phe	Gly	Tyr 140	Met	Gly	Glu	Ala		
Gly 145	Ile	Met	Ala	Ala	Trp 150	Pro	Ala	Phe	Ile	11e 155	Gly	Сув	Leu	Ala	Trp 160	•	
Val	Tyr	Met	Ile	Tyr 165	Glu	Leu	Tyr	Ala	Gly 170	Glu	Gly	Lys	Ser	Ala 175	Cys		_
Asn	Thr	Ala	Ser 180	Pro	Ala	Val	Gln	Ser 185	Ala	Tyr	Asn	Thr	Met 190	Met	Tyr		
Ile	Ile	Val 195	Phe	Gly	Trp	Ala	Ile 200	Tyr	Pro	Val	Gly	Tyr 205		Thr	Gly	·	
Tyr	Leu 210	Met	Gly	Asp	Gly	Gly 215	Ser	Ala	Leu	Asn	Leu 220	Asn	Leu	Ile	Tyr		٠.
Asn 225	Leu	Ala	Asp	Phe	Val 230	Asn	Lys	Ile	Leu	Phe 235	Gly	Leu	Ile	Ile	Trp 240		
Asn	Val	Ala	Val	Lys 245	Glu	Ser	Ser	Asn	Ala 250	,							
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<22: <22: <22: <22:	1> ( 2>		. (75) eorh		sin v	varia	ant i	Erom	pcr	clor	ne Mi	340ms	, q;ō	GenBa	ank #	AF34	19989
<40	0> 3	36															
atg	ggt	aaa								_	att Ile	_					48
											gat Asp						96

			•														
					act Thr												144
					aga Arg												192
					act Thr 70												240
					gag Glu												288
					aca Thr						_	_					336
					aca Thr								Lys		Leu		384 <sup>-</sup>
					gtt Val												432
	Ile				tgg Trp 150												480
					gaa Glu												528
					gct Ala	_			_			Thr					576
					tgg Trp												624
					ggt Gly												672
aac Asn 225	ctt Leu	gct Ala	gac Asp	ttt Phe	gtt Val 230	aac Asn	aag Lys	aat Asn	cta Leu	ttt Phe 235	ggt Gly	tta Leu	att Ile	ata Ile	tgg Trp 240	•	720

aat gtt gct gtt aaa gaa tct tct aat gct

750

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 <210> 37 <211> 250 <212> PRT Naturally occurring gamma proteobacterium <400> 37 Met Gly Lys Leu Leu Ile Leu Gly Ser Val Ile Ala Leu Pro Thr 5 Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45 Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 75 Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110 Ile Leu Ala Ala Ala Thr Asn Val Ala Ala Gly Leu Phe Lys Lys Leu 115 120 Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala ·· 130 135 Gly Ile Met Asn Ala Trp Gly Ala Phe Val Ile Gly Cys Leu Ala Trp 150 155

Va1	Tyr	Met	Ile	Туг 165	Glu	Leu	Trp	Ala	Gly 170	Ģlu	Gly	Lys	Ala	Ala 175	Cys	
Asn	Thr	Ala	Ser 180	Pro	Ala	Val	G1n	Ser 185	Ala	Tyr	Asn	Thr	Met 190	Met	Tyr	
Ile	Ile	Ile 195	Phe	Gly	Trp	Ala	Ile 200	Tyr	Pro	Val	Gly	<b>Tyr</b> 205	Phe	Thr	Gly	•
Tyr	Leu 210	Met	Gly	Asp	Gly	Gly 215	Ser	Ala	Leu	Asn	Leu 220	Asn	Leu	Ile	Tyr	
Asn 225	Leu.	Ala	Asp	Phe	Val 230	Asn	Lys	Asn	Leu	Phe 235	Gly	Leu	Ile	Ile	Trp 240	
Asn	Val	Ala	Val.	Lys 245	Glu	Ser	Ser	Asn	Ala 250		. <del>-</del>					
<210 <211 <212 <213	L> [ 2> [	38 750 DNA Natur	ally	, occ	urri	ng g	yamma	a pro	oteok	pacte	erium	ı.				
<220 <221 <222 <223	L> ( }>	CDS (1) Prote			sin v	varia	int f	rom	pcr	clor	ne ME	340m]	L2; (	SenBa	nnk #	AF34999
<400	)> 3	38											•			
		aaa Lys														48
		gca Ala										Tyr				96
		tgg Trp 35														144
		gaa														192

	50					5,5				60				
_				gtt Val				_			_		-	240
_		_		att Ile 85	-				-					288
	_			cta Leu		_				_				336
			_	gca Ala			_	_						384
	-			ctt Leu										432
		_	_	gca Ala			_							480
-		_		tat Tyr 165	_									528
		_	_	cct Pro	_				_					576
				ggt Gly										624
				gac Asp										672
		_	_	ttt Phe	_		-							720
	_	_	_	aaa Lys 245	_				-			٠		750

<210> 39

<211> 250 <212> PRT

<213> Naturally occurring gamma proteobacterium

<400> 39

Met Gly Lys Leu Leu Arg Ile Leu Gly Ser Val Ile Ala Leu Pro Thr 1 5 10 15

Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 30

Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu
100 105 110

Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Trp Ala Gly Glu Gly Lys Ser Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Tyr 180 185 190

														•			
	lle	Ile	Ile 195	Val	Gly	Trp	Ala	11e 200	Tyr	Pro	Val	Gly	Tyr 205	Phe	Thr	Gly	
	Tyr	Leu 210	Met	Gly	Asp	Gly	Gly 215	Ser	Ala	Leu	Asn	Leu 220	Asn	Leu	Ile	Tyr	
	Asn 225	Leu	Ala	Asp	Phe	Val 230	Asn	Lys	Ile	Leu	Phe 235	Gly	Leu	Ile	Ile	Trp 240	
	Asn	Val	Ala	Val	Lys 245	Glu	Ser	Ser	Asn	Ala 250					f	,	
	<210 <211 <212 <213	L> 7 2> I	10 750 ONA	calls	z oce	nirri	ina a	ramma	nro	oteol	oact.	eriur	n				
	<220 <221 <222	)> L> ( 2> ·	CDS	. (75(	) )				<del>-</del>					n5; (	GenBa	ank #AF3	349991
	<b>-</b> 400	)> 4	10									•					
	atg	ggt	aaa									att Ile					48
												gat Asp					96
		Phe										tct Ser					144
												aaa Lys 60				act Thr	192
-												cat His					240
												act Thr					288

WO 01/83701 PCT/US01/14394

				85					90					95			
		tgg Trp		Leu													336
		gct Ala 115	_	_			_	_									384
	_	ggt Gly			_	-										. •	432
		atg Met															480
		atg Met														•	528
		gca Ala			_												576
		gtc Val 195															624
		atg Met															672
		gct Ala	_	Phe	_	Asn	-	Ile		Phe	Gly						720
		gct Ala															750
-014																	
<210 <211 <212 <213	l> 2 2> 1	41 250 PRT Natur	rally	y oc	urri	ing g	-	a pro	oteol	pacte	eriur	n. ··					
<400	)> 4	41													• -		
Met 1	Gly	Lys	Leu	Leu 5	Leu	Ile	Leu	Gly	Ser 10	Val	Ile	Ala	Leu	Pro 15	Thr		
				-													

Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 30

Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala 180 185 190

Ile Ile Val Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly
195 200 205

Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220

225			2110	230		<b>-</b> 1.2		204	235		204		110	240	
Asn Val	Ala		Lys 245	Glu	Ser	Ser	Asn	A1a 250		,					
<211> '<212> 1	42 750 DNA Natura	ally	occ	urri	ing (	gamma	a pro	oteol	pacte	eriu	n				
<222>	CDS (1) Prote			sin v	varia	ant i	Erom	pcr	clo	ne M	B <b>1</b> 001	n7; (	GenB	ank	#AF349992 <sub>.</sub>
<400> 4 atg ggt Met Gly 1				_				_	_		_				
ttt gct Phe Ala	Ala						-	_	-	-				_	96
tct ttt Ser Phe					_	_						_			144
ttt gtt Phe Val 50															192
gta tct Val Ser 65							_								240
aga ggg Arg Gly	_	ľrp		_			_	_			_				288
att gat Ile Asp	Trp I				_						_				
att ctt Ile Leu															384

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp

115	1	120	125	
		ett gtg ttt ggt t Leu Val Phe Gly T 1	-	
		gca ttc att att g Ala Phe Ile Ile G 155		
_	<del>-</del>	tat gct gga gaa g Tyr Ala Gly Glu G 170	-,	
	Pro Ser Val G	caa tca gct tac a Gln Ser Ala Tyr A 185		
	Gly Trp Ala I	att tat cct gta g Ile Tyr Pro Val G 200		
		cca gct ctt aac t Ser Ala Leu Asn L 2		
		aag att cta ttt g Lys Ile Leu Phe G 235		
aat gct gct gtt Asn Ala Ala Val	_			750
<210> 43 <211> 250 <212> PRT <213> Naturall	y occurring ga	amma proteobacter	ium	
<400> 43				
Met Gly Lys Leu 1	Leu Leu Ile L 5	Leu Gly Ser Val I 10	le Ala Leu Pro 15	Thr
Phe Ala Ala Gly	Gly Gly Asp L	Leu Asp Ala Ser A 25	sp Tyr Thr Gly 30	Val
Ser Phe Trp Leu 35		Ala Leu Leu Ala S 10	er Thr Val Phe 45	Phe

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Tyr Ala Gly Glu Gly Lys Ser Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ser Val Gln Ser Ala Tyr Asn Thr Met Met Ala 180 185 190

Ile Ile Val Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly
195 200 205

Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240-

Asn Ala Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 44 <211> 750 <212> DNA <213> Naturally occurring gamma proteobacterium																	
<22	1> ( 2>	CDS (1). Prot	•	•	sin ¬	varia	ant :	from	pcr	clo	ne M	B1001	m9; (	GenB	ank #	AF349	993
atg		aaa										gca Ala					48
									-	_	_	tac Tyr		-	gtt. Val		96
						_	_			_		act Thr 45	_				144
												aca Thr					192
								_				tac Tyr	_		_	:	240
												gta Val					288
												gaa Glu				;	336
			Ala									ttt Phe 125				;	384
												atg Met					432
												tgt Cys				,	480

145	150	<b>)</b> ·	155		160
			t gga gaa gga a Gly Glu Gly 170		
			a gct tac.aac er Ala Tyr Asn 5		
			t cct gta ggt r Pro Val Gly		
_			a ctt aac tta a Leu Asn Leu 220		
		. Asn Lys Il	et cta ttt ggt e Leu Phe Gly 235		
	gtt aaa gaa Val Lys Glu 245				750
<210> 45 <211> 250 <212> PRT <213> Natu:	rally occuri	ing gamma p	proteobacterium	n	
<400> 45					•
Met Gly Lys 1	Leu Leu Leu 5	lle Leu Gl	y Ser Val Ile 10	Ala Leu Pro 15	Thr
Phe Ala Ala	Gly Gly Gly 20	Asp Leu As 25	p Ala Ser Asp	Tyr Thr Gly	Val
Ser Phe Trp 35	Leu Val Thr	Ala Ala Len 40	u Leu Ala Ser	Thr Val Phe 45	Phe
Phe Val Glu 50	Arg Asp Arg	Val Ser Ala 55	a Lys Trp Lys 60	Thr Ser Leu	Thr
Val Ser Gly 65	Leu Val Thr 70	Gly Ile Ala	a Phe Trp His 75	Tyr Met Tyr	Met 80

WO 01/83701 PCT/US01/14394

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Ile Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Trp Ala Gly Glu Gly Lys Ser Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Tyr 180 185 190

Ile Ile Ile Phe Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly
195 200 205

Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 46

<211> 750

<212> DNA

<213> Naturally occurring gamma proteobacterium

<220>

<221> CDS <222> (1)(75 <223> Proteorh		from pcr clo	one MB100m10;	GenBank #AF34999
	tta ctg ata tta Leu.Leu Ile Leu 5		_	
	ggc ggt gac ctt Gly Gly Asp Leu	-	=	
	gtt aca gct gct Val Thr Ala Ala 40			
	gat aga gtt tct Asp Arg Val Ser 55			
	gtt act ggt att Val Thr Gly Ile 70		-	_
	att gaa act ggt Ile Glu Thr Gly 85	-		
	cta aca gtt cct Leu Thr Val Pro			
	gca act aat gtt Ala Thr Asn Val 120	l Ala Gly Ser		
	ctt gtt atg ctt Leu Val Met Leu 135			
	gct tgg cct gca Ala Trp Pro Ala 150		Gly Cys Leu	
	tat gaa cta tgg Tyr Glu Leu Trg 165			
aat act gca agt	cct gct gta cag	g tca gct tac	e aac aca atg	atg tat 576

									•								
Asn	Thr	Ala	Ser 180	Pro	Ala	Val	Gln	Ser 185	Ala	Tyr	Asn	Thr	Met 190	Met	Tyr		
			_			_				_	ggt Gly					6	24
											cta Leu 220						72
			_		_		_				ggt Gly					7	20
	_	_	_	aaa Lys 245	_				_						٠.	7	50

<210> 47

<211> 250

<212> PRT

<213> Naturally occurring gamma proteobacterium

<400> 47

Met Gly Lys Leu Leu Leu Gly Ser Val Ile Ala Leu Pro Thr
1 5 15

Phe Ala Ala Gly Gly Gly Asp Leu Asp Ala Ser Asp Tyr Thr Gly Val 20 25 30

Ser Phe Trp Leu Val Thr Ala Ala Leu Leu Ala Ser Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Arg Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Val Thr Gly Ile Ala Phe Trp His Tyr Met Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Glu Thr Gly Asp Ser Pro Thr Val Phe Arg Tyr-85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Leu Ile Cys Glu Phe Tyr Leu

110

Ile Leu Ala Ala Ala Thr Asn Val Ala Gly Ser Leu Phe Lys Lys Leu 115 120 125

Leu Val Gly Ser Leu Val Met Leu Val Phe Gly Tyr Met Gly Glu Ala 130 135 140

Gly Ile Met Ala Ala Trp Pro Ala Phe Ile Val Gly Cys Leu Ala Trp 145 150 155 160

Val Tyr Met Ile Tyr Glu Leu Trp Ala Gly Glu Gly Lys Ser Ala Cys 165 170 175

Asn Thr Ala Ser Pro Ala Val Gln Ser Ala Tyr Asn Thr Met Met Tyr 180 185 190

Ile Ile Ile Val Gly Trp Ala Ile Tyr Pro Val Gly Tyr Phe Thr Gly
195 200 205

Tyr Leu Met Gly Asp Gly Gly Ser Ala Leu Asn Leu Asn Leu Ile Tyr 210 215 220

Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile Trp 225 230 235 240

Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 48

<211> 753

<212> DNA

<213> Naturally occurring gamma proteobacterium

<220>

<221> CDS

<222> (1)..(753)

<223> Proteorhodopsin variant from pcr clone PALB1; GenBank #AF349995

<400> 48

atg ggt aaa tta tta ctg ata tta ggt agt gct att gca ctt cca tca

Met 1	Gly	Lys	Leu	Leu 5	Leu	Ile	Leu	Gly	Ser 10	Ala	Ile	Ala	Leu	Pro 15	Ser	
								gat Asp 25								96
								atg Met								144
								gct Ala								192
								gct Ala								240
aga Arg	ggt Gly	gtt Val	tgg Trp	ata Ile 85	gat Asp	act Thr	ggt Gly	gat Asp	aca Thr 90	cca Pro	aca Thr	gta Val	ttt Phe	aga Arg 95	tat Tyr	288
								tta Leu 105								336
								gct Ala								384
cta Leu	gct Ala 130	ggt Gly	tca Ser	tta Leu	gta Val	atg Met 135	tta Leu	ggt Gly	gct Ala	gga Gly	ttt Phe 140	gca Ala	ggc Gly	gaa Glu	gct Ala	432
								ttc Phe								480
								atg Met								528
								tct Ser 185								576
								tat Tyr							ggt- Gly	624
tac	cta	atg	agt	ggt	gac	ggt	gta	tac	gct	tca	aac	tta	aac	ctt	ata	672

Tyr Leu Met	Ser Gly Asp	Gly Val Tyr 215	Ala Ser Asn 220		u Ile
		gtt aac aag Val Asn Lys			
		gaa tct tct Glu Ser Ser			753
<210> 49 <211> 251 <212> PRT <213> Natur	rally occurr	ing gamma pro	oteobacteriu	m	•
<400> 49					
Met Gly Lys 1	Leu Leu Leu 5	Ile Leu Gly	Ser Ala Ile 10	Ala Leu Pr 15	
Phe Ala Ala	Ala Gly Gly 20	Asp Leu Asp 25	Ile Ser Asp	Thr Val Gl	y Val
Ser Phe Trp 35	Leu Val Thr	Ala Gly Met	Leu Ala Ala	Thr Val Ph 45	e Phe
Phe Val Glu 50	Arg Asp Gln	Val Ser Ala 55	Lys Trp Lys 60	Thr Ser Le	u Thr
Val Ser Gly 65	Leu Ile Thr 70	Gly Ile Ala	Phe Trp His	Tyr Leu Ty	r Met 80
Arg Gly Val	Trp Ile Asp 85	Thr Gly Asp	Thr Pro Thr	Val Phe Ar	g Tyr
Ile Asp Trp	Leu Leu Thr	Val Pro Leu 105	Gln Met Val	Glu Phe Ty	r Leu
Ile Leu Ala 115	Ala Cys Thr	Ser Val Ala 120	Ala Ser Leu	Phe Lys Ly 125	s Leú <sup>.</sup>

Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala

135 140 130 Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Leu Gly Met Ala Gly Trp 150 155 Leu Tyr Met Ile Tyr Glu Leu His Met Gly Glu Gly Lys Ala Ala Val 165 170 Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Lys 185 190 ... 180 Ile Ile Val Ile Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 200 205 195 Tyr Leu Met Ser Gly Asp Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235 Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 <210> 50 <211> 753 <212> DNA <213> Naturally occurring gamma proteobacterium <220> <221> CDS <222> (1)..(753) <223> Proteorhodopsin variant from pcr clone PALB2; GenBank #AF349996 <400> 50 atg ggt aaa tta tta ctg ata tta ggt agt gct att gca ctt cca tca 48 Met Gly Lys Leu Leu Ele Leu Gly Ser Ala Ile Ala Leu Pro Ser 10 96 ttt get get get ggt gge gat eta gat ata agt gat act gtt ggt gtt Phe Ala Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20 25 30 144 tca ttc tgg ctg gtt aca gct ggt atg tta gcg gca act gtg ttc ttt

Ser	Phe	Trp 35	Leu	Val	Thr	Ala	Gly 40	Met	Leu	Ala	Ala	Thr 45	Val	Phe	Phe	
					caa Gln											192
					act Thr 70											240
_		_			gat Asp			_				-				288
					act Thr											336
					aca Thr											384
					gta Val		Leu									432
					tta Leu 150											480
					gag Glu											528
					gct Ala											576
					tgg Trp											624
					gaa Glu							Leu				672
					ttt Phe 230											720
tgg	aat	gtt	gct	gtt	aaa	gaa	tct	tct	aat	gct						753

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 51

<211> 251

<212> PRT

<213> Naturally occurring gamma proteobacterium

<400> 51

Met Gly Lys Leu Leu Leu Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser 1 5 10 15

Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20 25 30

Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Gln Val Ser Ala Glu Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125

Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 140

Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp-145 150 155 160

Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val

165 170 175

Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180 185 190

Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly
195 200 205

Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220

Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235. 240

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

. . . . . .

<210> 52

<211> 753

<212> DNA

<213> Naturally occurring gamma proteobacterium

<220>

<221> CDS

<222> (1)..(753)

<223> Proteorhodopsin variant from pcr clone PALB5; GenBank#AF349997

<400> 52

atg ggt aaa tta tta ctg ata tta ggt agt gct att gca ctt cca tca 48
Met Gly Lys Leu Leu Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser
1 5 10 15

ttt gct gct gct ggt ggc gat cta gat ata agt gat act gtt ggt gtt

Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val

20 25 30

tca ttc tgg ctg gtt aca gct ggt atg tta gcg gca act gtg ttc ttt

Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe

35 40 45

ttt gta gaa aga gac caa gtc agc gct aag tgg aaa act tca ctt act

192
Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr

50

55

60

gta tct ggt tta att act ggt ata gcc ttt tgg cat tat ctc tat atg 240

Val 65	Ser	Gly	Leu	Ile	Thr 70	Gly	Ile	Ala	Phe	Trp 75	His	Tyr	Leu	Tyr	Met 80		
				ata Ile 85													288
				tta Leu													336
att Ile	ctt Leu	gct Ala 115	gct Ala	tgt Cys	aca Thr	aat Asn	gtt Val 120	gct Ala	gct Ala	tca Ser	tta Leu	ttt Phe 125	aag Lys	aag Lys	ctt Leu	•	384
cta Leu	gct Ala 130	ggt Gly	tca Ser	tta Leu	gta Val	atg Met 135	tta Leu	ggt Gly	gct Ala	gga Gly	ttt Phe 140	gca Ala	ggc Gly	gaa Glu	gct Ala		432
				gta Val											tgg Trp 160		480
				tat Tyr 165													528
				cct Pro													576
att Ile	att Ile	gtt Val 195	gtt Val	gga Gly	tgg Trp	gca Ala	att Ile 200	tat Tyr	cct Pro	gct Ala	gga Gly	tat Tyr 205	gct Ala	gct Ala	ggt Gly		624
				ggc Gly													672
				gac Asp													720
				gtt Val 245								<b>≠</b> aust — vari				•.	753

<sup>&</sup>lt;210> 53

<sup>&</sup>lt;211> 251 <212> PRT

<sup>&</sup>lt;213> Naturally occurring gamma proteobacterium

<400> 53

Met Gly Lys Leu Leu Leu Gly Ser Ala Ile Ala Leu Pro Ser 1 5 10 15

Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20 25 30

Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Cys Thr Asn Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125

Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 140

Gly Leu Ala Pro Val Trp Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 150 155 160

Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 175

Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180 185 190

Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly

•		
195	200	205

Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220

Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235 240

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 54 <211> 753 <212> DNA

<213> Naturally occurring gamma proteobacterium

<220>

<221> CDS

<222> (1)..(753)

<223> Proteorhodopsin variant from pcr clone PalB7; GenBank #AF349999

ttt gct gct ggt ggc gat cta gat ata agt gat act gtt ggt gtt

Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val

20 25 30

tca ttc tgg ctg gtt acg gct ggt atg tta gcg gca act gta ttc ttt

Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe

35

40

45

ttt gta gaa aga gac caa gtc agc gct aag tgg aaa act tca ctt act

Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr

50 55 60

gta tct ggt tta att act ggt ata gct ttt tgg cat tat ctc tac atg
Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met
65 70 75 80

aga ggt gtt tgg ata gat act ggt gat aca cca aca gta ttt aga tat

288
Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr

85
90
95

att gat tgg tta tta act gtt cca tta caa atg gtt gag ttc tat cta 336

The Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115    cta gct ggt tca ttg gta atg tta ggt gct gga tct gca ggc gaa gct Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Ser Ala Gly Glu Ala 130    gga tta gct cct gta tta cct gct ttc att att ggt atg gct gga tgg Gly Leu Ala Pro Val Leu Pro Ala Phe IIe IIe Gly Met Ala Gly Trp 160    tta tac atg att tat gag cta tat atg ggt gaa ggt agg gct gct gct gta Leu Tyr Met IIe Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 175    agt act gca agt cct gct gtt aac tct gca tac aac gca atg atg atg Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 185    att att gtt gtt gga tgg gca att tat cct gct gga tac gct gct ggt 186    att att gtt gtt gga tgg gca att tat cct gct gga tac gct gct ggt IIe IIe Val Val Gly Trp Ala IIe Tyr Pro Ala Gly Tyr Ala Ala Gly 195    tac cta atg ggt ggc gaa ggt gta tac gct tca aac tta aac ctc ata Tyr Leu Met Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu IIe 210    tat aac ctt gct gac ttt gtt aac aag att cta ttt ggt ttg atc att Tyr Asn Leu Asn Leu IIe 220    tat aac ctt gct gac ttt gtt aac aag att cta ttt ggt ttg atc att Tyr Asn Leu Ala Asp Phe Val Asn Lys IIe Leu Phe Gly Leu IIe IIe 220    125    126    127    128    129    120    121    122    123    124    125    126    127    128    128    129    125    126    127    128    128    129    120    121    122    123    124    125    125    126    127    128    128    129    120    121    122    123    124    125    125    126    127    128    128    129    120    120    121    122    123    124    125    126    127    128    129    120    120    121    122    123    124    125    126    127    128    129    120    120    121    122    123    124    125    126    127    128    129    120    121    122    123    124    125    126    127    128    129    120    121    122    123    124    125    126    127    128    129    120    120    121    122    123    124    125    126    127    128    129    120    120    121    122    123    124    125    126    127    128    129    120    120    120																		
The Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115    cta gct ggt tca ttg gta atg tta ggt gct gga tct gca ggc gaa gct Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Ser Ala Gly Glu Ala 130    gga tta gct cct gta tta cct gct ttc att att ggt atg gct gga tgg Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 160    tta tac atg att tat gag cta tat atg ggt gaa ggt aag gct gct gta Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 175    agt act gca agt cct gct gtt aac tct gca tac aac gca atg atg atg Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180    att att gtt gtt gga tgg gca att tat cct gct gga tat gct gct gtf Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195    tac cta atg ggt ggc gaa ggt gta tac gct tca aac tta ac ctc ata Tyr Leu Met Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210    220    tac act gct gac ttt gtt aac aag att cta ttt ggt ttg atc att Tyr Asn Leu Asn Leu Ile 225    230      **Zet gaat gtt gct gtt aac gaa tct tct aat gct tra ac ctr gct gat tra acc ctr gct gac ttt gtt aac aag att cta ttt ggt ttg atc att Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 240    240    *Zet gaat gtt gct gtt aac gaa tct tct aat gct Tyr Asn Leu Ala Val Val Lys Glu Ser Ser Asn Ala 250    *Zet gaat gtt gct gtt aac gaa tct tct aat gct Tyr Asn Val Ala Val Val Lys Glu Ser Ser Asn Ala 250    *Zet Tyr Asn Val Ala Val Val Lys Glu Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Leu Phe Gly Leu Ile Ile 240    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala Gly Ser Ser Asn Ala 250    *Zet Ser Ala	Ile	Asp	Trp		Leu	Thr	Val	Pro		Gln	Met	Va1	Glu		Tyr	Leu		
Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Ser Ala Gly Glu Ala 130  gga tta gct cct gta tta cct gct ttc att att ggt atg gct gga tgg Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145  tta tac atg att tat gag cta tat atg ggt gaa ggt aag gct gct gta Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165  agt act gca agt cct gct gtt aac tct gca tac aac gca atg atg atg Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 185  att att gtt gtt gga tgg gca att tat cct gct gga tat gct gct gt Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195  tac cta atg ggt ggc gaa ggt tac gct cta acc tta acc gct ca acc tta acc ctc ata Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210  tat aac ctt gct gac ttt gtt aac aag att cta ttt ggt tg atc att Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225  4210			Āla					Val					Phe					384
GIY Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 150    tta tac atg att tat gag cta tat atg ggt gaa ggt aag gct gct gta Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 175    agt act gca agt cct gct gtt aac tct gca tac aac gca atg atg atg Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180    att att gtt gtt gga tgg gca att tat cct gct gga tat gct gct ggt Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Ala Gly 195    tac cta atg ggt ggc gaa ggt gta tac gct tca aac tta aac ctc ata Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210    tat aac ctt gct gct gac ttt gtt aac aag att cta ttt ggt ttg atc att Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225    **210		Ala					Met					Ser						432
Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 175  agt act gca agt cct gct gtt aac tct gca tac aac gca atg atg atg Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180  att att gtt gtt gga tgg gca att tat cct gct gga tat gct ggt ggt Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Ala Gly 195  tac cta atg ggt ggc gaa ggt gta tac gct tca aac tta aac ctc ata Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210  tat aac ctt gct gac ttt gtt aac aag att cta ttt ggt ttg atc att Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225  tgg aat gtt gct gtt aaa gaa tct tct aat gct Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245																		

<212> PRT

<213> Naturally occurring gamma proteobacterium

<400> 55

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Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val

25

30

Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe 35 40 45

Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr 50 55 60

Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125

Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Ser Ala Gly Glu Ala: 130 135 140

Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 150 155 160

Leu Tyr Met Ile Tyr Glu Leù Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 175

Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180 185 190

Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly
195 200 205

Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220

Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile

225 230 235 240

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

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<220> <221> <222> <223>	CDS (1). Prot			sin v	varia	ant d	Erom	pcr	clor	ne Pa	a1B6	; Gei	nBanl	c # 29	F349998
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ttt gc Phe Al															96
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ttt gt Phe Va 50															192
gta tc Val Se 65	t ggt r Gly	tta Leu	att Ile	act Thr 70	ggt Gly	ata Ile	gct Ala	ttt Phe	tgg Trp 75	cat His	tat Tyr	ctc Leu	tat Tyr	atg Met 80	240
aga gg Arg Gl	_			-			_								288
att ga Ile As															336
att ct Ile Le					Asn		Ala			Leu		Lys			384

125

120

•									
				tta Leu					432
				gct Ala					480
				tat Tyr					528
				aac Asn					576
				att Ile 200					624
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				aac Asn					720
				tct Ser			-	,	753

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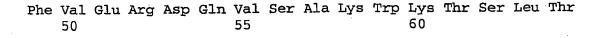
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<400> 57

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Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe 35 40 45



Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 85 90 95

Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 110

Ile Leu Ala Ala Cys Thr Asn Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125

Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 140

Gly Leu Ala Pro Val Trp Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 150 155 160

Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 175

Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Val 180 185 190

Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly
195 200 205

Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220

Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235 240

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

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ttt gc Phe Al	-	_	-				_					-				96
tca tt Ser Ph															1	44
ttt gt Phe Va 50	1 Ğlu	_	_		-	_	_								1	92.
gta tc Val Se 65															2	40
aga gg Arg Gl	_			_			_				_				2	88
att ga Ile As									_						3	36
att ct Ile Le															3	84
cta gc Leu Al 13	a Gly														4	32
gga tt Gly Le 145															4	80

50

						cta Leu										528	
						gtt Val										576	
						gca Ala										624	
						ggt Gly 215										672	
			_	_		gtt Val		_								720	
		_	_	_		gaa Glu				_			s.			~; <b>753</b>	•
<210 <211 <212 <213	L> 2 2> 1		rally	, oc	curr	ing g	yanma	a pro	oteok	pacte	eria						
<400	)> !	59															
Met 1	Gly	Lys	Leu	Leu 5	Leu	Ile	Leu	G1y	Ser 10	Ala	Ile	Ala	Leu	Pro 15	Ser		
Phe	Ala	Ala	Ala 20	Gly	Gly	Asp	Leu	Asp 25	Ile	Ser	Asp	Thr	Val 30	Gly	Val		
Ser	Phe	Trp	Leu	Val	Thr	Ala	Gly	Met	Leu	Ala	Ala	Thr	Va1	Phe	Phe		

. 45

60

40

55

Phe Val Glu Arg Asp Gln Val Ser Ala Lys Trp Lys Thr Ser Leu Thr

Val Ser Gly Leu Ile Thr Gly Ile Ala Phe Trp His Tyr Leu Tyr Met 65 70 75 80

Arg Gly Val Trp Ile Asp Thr Gly Asp Thr Pro Thr Val Phe Arg Tyr 90 85 Ile Asp Trp Leu Leu Thr Val Pro Leu Gln Met Val Glu Phe Tyr Leu 100 105 Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 140 Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 150 155 160 Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Met 180 185 190 Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195 200 205 Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 Tyr Asn Leu Ala Asp Leu Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235

Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 250

<210> 60

<211> 753

<212> DNA

<213> Naturally occurring gamma proteobacteria

<220>

<221> CDS

<222> (1)..(753)

PCT/US01/14394

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	_	_				-		_			_		gtt Val 30		-	96
		_		_			-	_			_		gtg Val			144
	_	-	_	_		-	_	_	_				tca Ser			192
-								_					ctc Leu		_	240
		_			_			_				-	ttc Phe	Arg		288
													ttc Phe 110		cta Leu	336
		_	_	-		_	_	_	_				aag Lys	_		384
													ggc Gly			432
													gct Ala			480
													gct Ala			528
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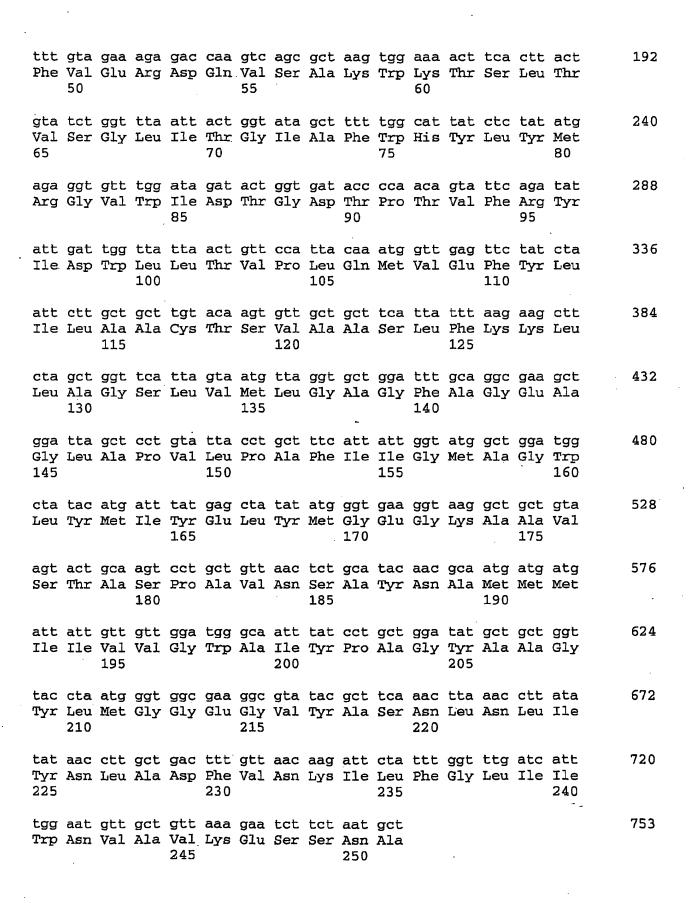
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			c gct tca aa r Ala Ser As 22	n Leu Asn	
		Val Asn Ly	g att cta tt s Ile Leu Ph 235		
tgg aat gtt Trp Asn Val		gaa tot to Glu Ser Se	-		753
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Phe Ala Ala	Ala Gly Gly 20	Asp Leu As 25	o Ile Ser As	p Thr Val	Gly Val
Ser Phe Trp 35	Leu Val Th	: Alà Gly Me 40	t Leu Ala Al	a Thr Val 45	Phe Phe
Phe Val Glu 50	Arg Asp Gli	ı Val Ser Ala 55	a Lys Trp Ly 60		Leu Thr
Val Ser Gly 65	Leu Ile Th	Gly Ile Al	a Phe Trp Hi 75	s Tyr Leu	Tyr Met 80
Arg Gly Val	Trp Ile Ası	Thr Gly As	o Thr Pro Th 90	r Val Phe	Arg Tyr 95
Ile Asp Trp	Leu Leu Th	Val Pro Le	u Gln Val Va 5	l Glu Phe 110	Tyr Leu

Ile Leu Ala Ala Cys Thr Ser Val Ala Ala Ser Leu Phe Lys Lys Leu 115 120 125 Leu Ala Gly Ser Leu Val Met Leu Gly Ala Gly Phe Ala Gly Glu Ala 130 135 Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 145 Leu Tyr Met Ile Tyr Glu Leu Tyr Met Gly Glu Gly Lys Ala Ala Val 165 170 Ser Thr Ala Ser Pro Ala Val Asn Pro Ala Tyr Asn Ala Met Met 180 185 Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195 200 205 Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 230 225 235 Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala 245 <210> 62 <211> 753 <212> DNA <213> Naturally occurring gamma proteobacterium <220> <221> CDS <222> (1)..(753) <223> Proteorhodopsin variant from pcr clone PalE6; GenBank#AF350002 <400> 62 atg ggt aaa tta tta ctg ata tta ggt agt gct att gca ctt cca tca 48 Met Gly Lys Leu Leu Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser 10

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							gta Val			144
							tca Ser			192
							ctc Leu		atg Met 80	240
							ttt Phe			288
			_		_	_	 ttc Phe 110			336
							aag Lys			384
							ggc			432
							gct Ala			480
							gct Ala			528
							atg Met 190			576
			_		_		gct Ala	-		624
							aac Asn			672

			ctt Leu														72	0
			gtt Val				-	•			_						75	3
	<210 <211 <212 <213	L> 2 2> 1	63 251 PRT Natur	cally	/ occ	curri	ing (	gamma	a pro	oteok	Dacte	erium	n					
	<400	)> (	53															
	Met 1	Gly	Lys	Leu	Leu 5	Leu	Ile	Leu	Gly	Ser 10	Ala	Ile	Ala	Leu	Pro 15	Ser		
	Phe	Ala	Ala	Äla 20	Gly	Gly	Asp	Leu	Asp 25	Ile	Ser	Asp	Thr	Val 30	Gly	Val		
	Ser	Phe	Trp 35	Leu	Val	Thr	Ala	Gly 40	Met	Leu	Ala	Ala	Thr 45	Val	Phe	Phe		
	Phe	Val	Glu	Arg	Asp	Gln	Val 55	Ser	Ala	Lys	Trp	Lys 60	Thr	Ser	Leu	Thr		
	Val 65	Ser	Gly	Leu	Ile	Thr 70	Gly	Ile	Ala	Phe	Trp 75	His	Tyr	Leu	Tyr	Met 80		
	Arg	Gly	Val	Trp	Ile 85	Asp	Thr	Gly	Asp	Thr 90	Pro	Thr	Val	Phe	Arg 95	тут	-	
	Ile	Asp	Trp	Leu 100	Leu	Thr	Val	Pro	Leu 105	Gln	Met	Val	Glu	Phe 110	Tyr	Leu		
	Ile	Leu	Ala 115	Ala	Cys	Thr	Ser	Val 120	Ala	Ala	Ser	Leu	Phe 125	Lys	Lys	Leu		
-	Leu	Ala 130	Gly	Ser	Leu	Val	Met 135	Leu	Gly	Ala	Gly	Phe 140	Ala	Gly	G1u	Ala		

Gly Leu Ala Pro Val Leu Pro Ala Phe Ile Ile Gly Met Ala Gly Trp 150 145 155 160 Leu Tyr Met Ile Tyr Glu Leu His Met Gly Glu Gly Lys Ala Ala Val 165 170 Ser Thr Ala Ser Pro Ala Val Asn Ser Ala Tyr Asn Ala Met Met Lys 180 185 Ile Ile Val Ile Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 200. Tyr Leu Met Ser Gly Asp Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220 Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 230 235 225 Trp Asn Val Ala Val Lys Glu Ser Ser Asn Ala <210> 64 <211> 753 <212> DNA <213> Naturally occurring gamma proteobacterium <220> <221> CDS <222> (1)..(753) <223> Proteorhodopsin variant from pcr clone PalE7; GenBank# AF350003 <400> 64 48 atg ggt aaa tta tta ctg ata tta ggt agt gct att gca ctt cca tca Met Gly Lys Leu Leu Leu Ile Leu Gly Ser Ala Ile Ala Leu Pro Ser 1 ttt get get get ggt gge gat eta gat ata agt gat aet gtt ggt gtt 96 Phe Ala Ala Gly Gly Asp Leu Asp Ile Ser Asp Thr Val Gly Val 20 25 144 tca ttc tgg ctg gtt aca gct ggt atg tta gcg gca act gtg ttc ttt Ser Phe Trp Leu Val Thr Ala Gly Met Leu Ala Ala Thr Val Phe Phe 35 40 45



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175

170

165

Ser	Thr	Ala	Ser	Pro	Ala	Val	Asn	Ser	Ala	Tyr	Asn	Ala	Met	Met	Met
			180					185		•			190		

Ile Ile Val Val Gly Trp Ala Ile Tyr Pro Ala Gly Tyr Ala Ala Gly 195 200 205

Tyr Leu Met Gly Gly Glu Gly Val Tyr Ala Ser Asn Leu Asn Leu Ile 210 215 220

Tyr Asn Leu Ala Asp Phe Val Asn Lys Ile Leu Phe Gly Leu Ile Ile 225 230 235 240